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Linking science to technology: harder than it seems for Third World

THERE is a moral, a lesson and a set of danger signals in the three articles on science policy in Latin America in this week's *Nature* (pages 472-475). The moral is that linking a country's scientific activities to its technological needs can, in practice, be very much harder than the familiar political rhetoric would often have us believe. The lesson comes from the Andean Pact countries, who have been able to demonstrate how cooperation in research can help overcome some of the problems resulting from an over-dependence on scientific and technical skills of industrialised nations.

The danger signals relate to the increasingly important role of foreign investment—rapidly replacing the multinational company as the bogeyman of development—in determining scientific and technological research policies, not only in content but also in form.

Each of these observations must be seen in relation to a basic premise: that, in the words of a major study of the instruments of Latin American science and technology policy financed by Canada's International Development Research Centre, "the development of indigenous science and technology capabilities [is] an essential condition for achieving a certain degree of autonomy in decision-making on industrial development."

The experiences of both Venezuela and Peru in putting flesh on the bare bones of such statements have implications for other developing countries. Venezuela, for example, has attempted to develop a centralised strategy for its research policy through the creation of a National Council for Science and Technology (CONICIT), a model adopted by several other countries in the region. But the explicit goals of this strategy have been undermined by the consequences of an economic policy which, in pursuing rapid industrialisation, has encouraged joint ventures between foreign multinational companies and local capital. The result has frequently been to stimulate, rather than restrain, the flow of foreign technology into the country, providing companies with little incentive to support an indigenous research and development capacity.

Peru's problems have been slightly different. Since taking power in 1968, the country's military rulers have attempted to pursue a strongly independent economic policy; this has included not only the nationalisation of basic industries, but also the rapid increase in research budgets (up by a factor of 10 since the late 1960s) and support for both public and private research institutes. Subsequent moves to centralise science planning, however, seem destined to be moulded, at least in part, by the economic measures being demanded by the International Monetary Fund; these have been made the condition for bailing Peru out of the plight caused among other things by high military spending and the declining world price of copper.

In both Venezuela and Peru, science and technology policy is increasingly coming to reflect the domestic role played by foreign capital. And the danger of this is that it

skews decision-making to favour research and development policies, for example those concentrating on capital rather than labour-intensive production techniques, that benefit the upper and intermediate groups in society but have little impact on the plight of the poorest. Even where a "basic needs" technology is simultaneously advocated, this lies open to charges of "buying-off" the demands of the latter for an equitable share in the fruits of development.

Such issues can no longer be ignored by those concerned about the role of science in the development process. Fifteen years ago, the argument that the path to development lay in the rapid transfer of scientific and technological knowledge to the Third World, led participants in a United Nations conference to list priorities for such transfer in a "World Plan of Action". Today it is accepted that limitations and constraints on the transfer process are such that this philosophy is no longer adequate; indeed these limitations and constraints are the main focus of consideration.

This shift, embodied in the agenda for next year's United Nations Conference on Science and Technology for Development (UNCSTD), itself implies recognition that science and technology lie at the very heart of the development process. In practice, it is also coming to be recognised that policy matters in these areas can no longer be legitimately left to groups of experts, scientific or otherwise, but can only be forged through the type of bargaining and negotiation conventionally limited to more overtly political areas of national and international diplomacy.

Here there are lessons to be learnt from the recent UN conference on Technical Co-operation Among Developing Countries (TCDC) in Buenos Aires last month. Despite gloomy forebodings for the conference which predicted that the "real issues" would disappear in a storm of political acrimony, the meeting seems to have turned out relatively successful. In particular, the developing nations were able to unite behind a coherent and well-thought out position; and their combined strength was sufficient to add considerable bite to the draft plan of action which had been submitted by the UN secretariat, for example giving them greater control over the allocation of technical development funds.

TCDC marked an important shift from confrontation to negotiation that other UN conferences, including UNCSTD, would do well to try to follow. Certainly there are common scientific and technological interests between rich and poor nations; but given that these reflect broader social goals, no-one should be surprised that there are also conflicts. How to make such conflict productive, rather than merely self-defeating, is one of the most important issues that UNCSTD will have to confront. Encouraging joint research projects between developing countries, for example, can both provide practical benefits and increase the negotiating strength of the countries concerned; and ultimately it is this which determines who gets what share of the cake. □



DNA: let the public choose

Susan Wright, a historian of science at the University of Michigan, has followed the recombinant DNA debate closely. Here she comments on the recent public discussion of the revised NIH containment guidelines.

MUCH attention is currently being given to the concept of 'public participation' in policy decisions on technical and scientific matters. In referring to decisions on recombinant DNA research during a commencement address at the University of Michigan, Joseph Califano, Secretary of Health, Education, and Welfare, emphasised the importance of due process: "This means decisions made democratically through wide consultation, not by special elites". The tension in that statement, between "democratic" procedure and "consultation" (the results of which may still be digested by an elite group), points to a problem itself. Technical policy-making bodies are older than the growth of interest in participatory forms of decision making. They reflect an earlier faith in delegation of responsibility to high-ranking civil servants and expert advisors.

The political distance between current demands for participation in policy decisions on science and technology and the actual composition and procedures of policy-making bodies is nicely illustrated by the arrangements adopted by the NIH for decisions on recombinant DNA research. The elite nature of those arrangements was a principal subject of testimony at a hearing on proposed revisions to the NIH guidelines held at the Department of Health, Education, and Welfare in September before a committee chaired by Peter Libassi, HEW general counsel. NIH officials have claimed that all sectors have contributed to decisions on the guidelines through hearings and through the publication for comment of the guidelines, proposed revisions, and an environmental impact statement. However, testimony on the proposed revisions shows that many sectors see those procedures largely as a means for legitimising decisions taken within biomedical research circles rather than for facilitating participation in decisions.

The proposed revisions to the guidelines (10 August, page 411) are substantial: lowering of containment levels for many classes of experiment; exemptions which would take many experiments out of the guideline's control; and delegation of important responsibilities from NIH to local biohazards committees. Under the changes, local committees would be given wide responsibilities for recombinant DNA experiments. At a time when recombinant DNA technology promises to become as important for industry as it is for the universities, the revisions are a major step towards dismantling the central controls established two years ago.

All those who testified in support of the revisions were scientists and administrators with close ties to biomedical and agricultural research. Their reasons for approval were generally in line with those given by NIH director Fredrickson in his introduction to the guidelines. Delegation of authority to local biohazard committees was seen as a step in the right direction.

Critics of the proposed revisions included scientists and lawyers with ties to state and local government, trade unions, and public interest groups as well as to the universities. A concern expressed repeatedly at the hearing was that the circle of participation in decision-making has been largely restricted to a small group of scientists with close ties to NIH. According to Philip Bereano, a lawyer and professor in the University of Washington's Social Management of Technology Program, NIH decision making has

been dominated by an "old-boy network"—a reflection, ultimately, of the basic conflict of interest of the NIH in attempting to regulate an activity that it also promotes. Bereano's claim was supported by data from an NSF-funded study of the composition of institutional biohazard committees (IBCs) reported by Nancy Pfund of Stanford University. Preliminary results show that the proportions of workers, students, and members without ties to the institutions doing the research averaged less than 2% and that almost 70% of the membership was drawn from scientists and administrators in such fields as microbiology, biochemistry and molecular genetics.

Witnesses noted that growing industrial interest in recombinant DNA means that the NIH advisory committee will be called on increasingly in the future to make policy recommendations with far-reaching social implications. Fredrickson's proposal to "broaden [the committee] modestly as needed for expertise" and "to include, perhaps, a dissenter from NIH policies" was seen as an inadequate response to those demands. In written and oral testimony, representatives of the Attorney General of New York State, the American Federation of Labor and Congress of Industrial Organisations, the Coalition for Responsible Genetic Research, the Environmental Defense Fund, and Friends of the Earth called for substantial expansion of the committee to include members of trade unions, public interest groups, public health organisations, and government agencies with potential regulatory jurisdiction. In addition, Friends of the Earth lawyer Richard Hartzman stated that the proposed changes were so sweeping that they required a new environmental impact statement.

The proposal to delegate responsibility for oversight to local institutions also received strong criticism. Pointing to violations of the guidelines that have occurred at Harvard Medical School and the University of California, San Francisco, commentators suggested that to respond to those events by enhancing rather than by checking responsibilities of local committees was inappropriate. "NIH proposes a form of enforcement which institutionalises conflict of interest and provides even less accountability for the Director of NIH and the IBCs than the previous guidelines," wrote Marcia Cleveland and Louis Slesin of the Natural Resources Defense Council. This matter is also of concern to Assistant Secretary of Labor Eula Bingham and Senator Adlai Stevenson, chairman of the Senate subcommittee on Science, Technology, and Space, both of whom have proposed retention of more centralised controls.

Critics have also claimed that the closed form of decision-making had affected the quality of the technical evidence used in reaching decisions. "There has been heavy reliance on unrefereed, unexamined reports of small committees meeting in private which are then presented as the consensus of the scientific community," said Jonathan King, associate professor of biology at MIT. He stated that the results from those meetings had been used in an "extraordinarily selective manner in which no arguments that countered or failed to support claims were either quoted or referred to."

How will the DHEW handle testimony on the revisions? Will a full environmental impact statement be necessary? Libassi after the hearing declined to say what he would do, but, echoing Califano's words, he did say that he thought the issues raised were "not ones to be decided by scientists." As to his own role: "A lawyer's skills can be applied in technical areas as well as procedural ones." Clearly, several government sectors and the public will follow closely how the sentiments of Libassi and Califano are translated in terms of expansion of the present circle of recombinant DNA decision-making. □

Where should LEP be built?

Europe's bid for a world lead in subnuclear physics in the 1990s brings CERN's future into question, writes **Robert Walgate**

EUROPEAN subnuclear physicists are brimming with enthusiasm for their next big project, LEP (see page 482), but building it will plunge CERN (the European organisation for nuclear research) into a hornet's nest of problems.

There is strong competition—tacit or not—between CERN and the West German laboratory DESY for the siting of LEP, a large electron-positron storage ring; and there is controversy over CERN costs, with large tax-free wages accounting for over half CERN's expenditure. The French, German, and British governments are insisting that the CERN budget be brought down from its present 600 million Swiss Francs (MSF) a year to 560 MSF by 1981, but LEP can only be built at reasonable speed (in under a decade) if CERN has some 600 MSF a year.

Meanwhile, the tacit international understanding that the next machines would be continental, with the USSR building a 3 TeV proton synchrotron, Europe building a 100 GeV electron-positron storage ring (LEP), and the US building Isabelle (a 400 GeV proton storage ring), has been violated with the news that the US is also looking at a LEP-like project. Burton Richter, the Nobel laureate who led the discovery of charm with LEP's progenitor, SPEAR, is reputed to be pressing the US government for an American LEP. And as the US Department of Energy is discussing collaboration with Japan on a number of research issues, including subnuclear physics, it is conceivable that Professor Richter will find Japanese money to build a LEP ahead of Europe despite US expenditure on Isabelle and Fermilab. The race is on, and as one CERN physicist put it "the first to the money will get LEP".

The future of LEP in Europe is confused by a number of factors, one of which is the rising eminence of the German laboratory DESY. "Four years ago we were nowhere", a DESY physicist told *Nature*. "Now we are on top, and physicists are clamouring to work here". The root cause is the realisation that electrons, particularly in collision with positrons, are the cleanest probes of the nature of matter on small scales. DESY is Europe's largest electron laboratory. Further, DESY has succeeded in building PETRA.

PETRA is the world's largest storage ring for electrons and positrons, with (at 19 GeV) twice the energy per beam of SPEAR (and its equivalent at DESY, DORIS). It will begin experiments on

22 October, eight months ahead of schedule, though it may take a month or two before its intensity reaches design levels. It will probe new quark combinations (like 'bottomonium') and most likely reveal new quarks ('top' is expected) and leptons. And with an experiment devised by Samuel Ting (co-winner with Burton Richter of the Nobel Prize for the discovery of the 'hidden charm' particle J/ψ) it will test the unification of the weak and electromagnetic forces proposed by Abdus Salam and Steven Weinberg. PETRA will give European subnuclear physics a world lead for five years or more.

Experience in building and running PETRA also gives DESY physicists a European lead in the design considerations for LEP. But there was not a single DESY representative on the 63-man CERN study of LEP, recently completed (CERN/ISR-LEP/78-17, the 'Blue book'). Professor Schopper, the director of DESY, argues that the best contribution that DESY could have made was to build PETRA, rather than to work on "a paper study"; and anyway the CERN team spent a long time at DESY, discussing the PETRA design. Schopper points out that several of the design points in the CERN study are a simple scaling-up of PETRA elements—the rf cavities, for example, and the vacuum tube. DESY physicists were of course spending 16 hours a day building PETRA, but the DESY absence from the study has also been interpreted as a political manoeuvre; so much so that the brief presence of several senior representatives of DESY, including the builder of PETRA, Gustav Voss, at the CERN summer study on LEP recently was seen as something of a thaw.

All this would matter little if it did not involve the future of CERN. The CERN budget is under pressure from its staff (some 4,000 people, mostly technicians) whose promotions have fallen to a low ebb, and who are, of course, keen to maintain salaries at their high level. (50% of the staff live in France, where they could earn only half the sums they cull from CERN; a study, called RESCO, is underway on the social conditions and remuneration of workers at CERN.) The budget is also under pressure from the Swiss Franc, in which CERN subscriptions and salaries are payable. The franc has strengthened from 12 SF to the £ in 1966 to 3 SF at present, quadrupling the British subscription over that



Who should CERN fear most: Chancellor Schmidt (left) or DESY director Herwig Schopper?

period. And the budget is under pressure from the other direction from the member states, who want to keep their expenditure on subnuclear physics in proportion to their other research costs. The CERN-built LEP would cost some 1000 MSF, but as a French physicist commented, "what will that mean in 10 years in pounds, or French francs, or even Deutsche marks?"

According to one influential physicist, the European Committee for Future Accelerators (ECFA), which has been charged by CERN members with producing a firm proposal for LEP, has in its hands a very delicate decision. The committee, he said, has to consider which course of action for LEP would preserve the best future for subnuclear physics in Europe. LEP at CERN's Geneva site might price itself out of the market. And CERN without LEP in the 1990s would be a dying laboratory. The question seems to be whether the focus for subnuclear physics in Europe should move for the 1990s from Geneva to Germany (though there are other contenders, notably France; only Italy has ruled itself out).

CERN defends itself strongly, pointing out that a new laboratory would need a new infrastructure, entailing extra cost; that LEP at Geneva would allow collisions between the electrons of LEP and the protons of the SPS, allowing an ultradeep probe of proton—and perhaps quark—structure; and

that the LEP tunnel, once built, could be used for many other purposes at CERN, with its complex of accelerators.

Over at DESY, Professor Schopper is at pains to protest that there is no competition between his laboratory and CERN; but other DESY physicists see it differently. "We are planning moves like a chess game", said one. And DESY can afford to sit tight for the moment: it is in a strong position, technically and politically. On the technical side, pressure from physicists for the highest possible energy favours DESY. High energy means large ring size to reduce the synchrotron radiation losses from the circulating electrons (and so keep down the power bill). But the CERN site is restricted by the Jura mountains to the north. DESY's presently favoured site, in a national park 40 km south of DESY near Hamburg, is not so restricted.

However, an early CERN study of a 100 GeV machine (LEP-100) was abandoned. The 'Blue book' argues that there will be many difficulties in handling electron beams at 100 GeV. The summer study concluded that "improving the energy [beyond 70 GeV] requires far more knowledge of a theoretical and technical nature before a realistic design may be settled upon"; but Schopper says that such conclusions are premature, and based on a too-pessimistic interpretation of work on the problem at DESY. In principle DESY should know, but it is not easy to disentangle physics from politics on this point.

The present half-cost LEP planned by CERN is a project for 70 GeV beams (LEP-70), with a possibility to upgrade to 100 GeV if superconducting rf power cavities become feasible. One of the key tasks for LEP will be to produce the intermediate vector bosons, which have masses and properties such that the neutral Z^0 could be produced with single beam energies of about 45 GeV, while the charged W^+ and W^- would need 80 GeV. The Higgs bosons, which are essential to the unification of the forces, and hold the key to the masses of the quarks and leptons, require the highest possible energy. LEP-70 is not therefore the most comfortable machine for physicists — 100 GeV would be better—although there will be a cornucopia of treasures on LEP-70 from the "resonance" in the interaction at the Z^0 , which should yield a thousand-fold increase in interaction rate.

ECFA is just beginning studies of the machine parameters for LEP, under the chairmanship of Professor Antonino Zichichi—who intends to be entirely "Galilean" in his considerations. In other words he wants his committee to come up with the physi-

cists' perfect proposal, without regard to politics. That study is due at the end of 1979 for presentation to ECFA and to CERN Council. It will be interesting to see whether it more nearly approaches LEP-70 or LEP-100. ECFA will then have to juggle with site and politics to come to its recommendation.

John Adams, CERN's machine-building director-general, said in Geneva recently that he would like physicists to reach agreement on a site "by the end of next year". He added "If the physicists can't decide where to put the thing they can't blame governments for delays". And there are further battles to be won at governmental level.

Germany lost the fusion project, JET, to the UK, and might welcome a new international laboratory. Professor Schopper insists that any extension of

DESY would be "fully international", with contributions from no country ruled out (including Japan). And the small countries at CERN will not be happy with the closure of CERN's low energy synchrocyclotron and the intersecting storage rings, as will be necessary if CERN is to build LEP within its 600 MSF ceiling. The Scandinavian countries, in particular, make good use of the synchrocyclotron for nuclear physics, and the intersecting storage rings are now ready for nuclear physics experiments with light nuclei.

CERN's problems with LEP are manifold, and it is no wonder that before John Adams gave his recent talk at CERN on "steering LEP" to an enthusiastic audience of physicists his colleagues told him, "And for God's sake be cheerful".

Robert Walgate

Argentina— where thought is a crime

DR CLAUDIO BERMANN, a professor of psychiatry held incommunicado in Cordoba prison in Argentina without specific charges since April 1976, was released last month, following pressure from the US National Academy of Sciences' Committee on Human Rights, the Israeli embassy in Buenos Aires, and international human rights groups.

Dr Bermann was released into the custody of an Israeli representative only when he was actually aboard the aircraft bound for Israel. On arrival in Israel, he avoided any formal contact with the Press, apparently fearing for the safety of fellow-prisoners still in custody. It was, however, possible to learn informally some interesting sidelights on the position of psychiatrists in Argentina, and, in particular, the reason why so many psychiatrists figure in the lists of political prisoners and 'disappeared persons'.

"Thought is a crime there," explained a friend of Dr Bermann's cynically. More particularly, psychiatry which, as a therapeutic discipline, aims at establishing the autonomy of the individual, is seen by the present regime as potentially subversive—an opinion which draws some substantiation from the fact that, until 1973, the Argentinian Federation of Psychologists did, as a matter of fact, have a large number of members whose political leanings were leftist or liberal. Freudian psychology is banned from the universities (where it is seen as a Marxist subversion unlike the Soviet Union where it is condemned for being anti-Marxist!), and, according to



"I keep thinking I'm two psychiatrists!"

another expatriate psychologist, university teaching in psychology goes no further than the writings of St Augustine and St Thomas Aquinas.

Furthermore, said the same psychologist, difficulties can arise in the therapeutic situation. A psychiatrist might attempt to treat a patient's anxieties by presenting the current regime as an unpleasant, unavoidable fact of life, which one must somehow learn to live with. Further there are reports of members of guerilla groups actually receiving treatment from psychiatrists without the psychiatrist actually knowing anything about this side of their activities—a proposition which seems at first glance somewhat unlikely, but which, within a group therapy context, is quite feasible.

Acting on suspicion of some such link between psychiatrists and guerillas, the Argentinian authorities have, according to Dr Bermann and his friends, been putting pressure on practising psychiatrists to betray their professional confidentiality, and, in some cases, it would appear that *agent-*

provocateurs have been used, posing as guerrillas in need of treatment, to determine the "loyalty" of the psychiatrists.

According to Dr Bermann, when first arrested, he was himself tortured in an attempt to establish a link between him and the guerrillas. When the authorities finally realised that he had no information to give them, he was put in a cell with other "political prisoners who knew nothing," from which the sounds of others being tortured could be heard.

Dr Bermann, as a professor and former director of a psychiatric clinic, ranked as an important prisoner—and as such was chosen as a kind of hostage when President General Jorge H. Videla visited Cordoba last year. Twenty such hostages were arrested—though as incommunicado prisoners they could do nothing to avert any possible attack on the presidential party. Nevertheless, their fate depended on the safety of the President and his entourage, according to an equation

which seems to be a peculiarly Argentinian way of assessing the value of its leading citizens.

If there was any attempt on the President's life, they were told, all 20 would be shot; if a General was killed, 14 would be shot, if a colonel, eight, and if any other official or policeman were killed, only two. So, by the new Argentinian mathematics, it would appear that one leading psychiatrist equals half a policeman!

Vera Rich

US producers fear ban on 2,4,5-trichlorophenol

SOME of the producers of 2,4,5-trichlorophenol fear that it might be banned in the US. The Environmental Protection Agency (EPA) has recently issued a notice of 'rebuttal presumption' against current and continued registration of trichlorophenol and its salts. The notice—the first of several which the agency intends to issue—says that they exceed "certain risk criteria".

The issuing of a rebuttal presumption is not, says the agency, notice of intent to ban the products. Such action would only be taken after careful evaluation of all the evidence had shown that their continued use would cause "unreasonable adverse effects to the environment". However, the notice does require that the users and manufacturers of trichlorophenol submit evidence to the EPA, proving that its continued use does not constitute a hazard.

This is not the first time that the EPA has reviewed these particular chemicals. 2,4,5-trichlorophenol is used in the manufacture of the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and of the bactericide, hexachlorophene. The reaction process used to make trichlorophenol produces an extremely toxic by-product, tetrachlorodibenzo-p-dioxin (TCDD). It is the presence of TCDD—a known teratogen and potential carcinogen—which is causing the current concern.

2,4,5-T has been reviewed recently by government committees in the UK and Australia; both review bodies pronounced the herbicide safe if used for the purposes for which it is intended. Manufacturers are less optimistic about its chances in the US.

Among the most recent evidence that the EPA will examine are two sets of documents relating to the safety of the manufacturing process. The first is the Italian Parliamentary Commission's

report of its investigation into the accident which occurred in the ICMESA trichlorophenol reactor near the Italian town of Séveso nearly two years ago. The second is the report of ICMESA's Swiss owners, Givaudan and Hoffman-La Roche, on their own research into the cause of the accident.

Their report is in reply to the commission, but it has been prepared especially for the EPA. The commission's report, which runs to several hundred pages, accuses Roche and Givaudan of being less than candid about their operations at ICMESA and of running an unsafe chemical plant. The commission also claims that ICMESA was producing trichlorophenol without an up-to-date 'fire precautions certificate'; that it failed to inform the Labour Inspectorate that it had begun production of trichlorophenol; that it failed to notify the authorities of the accident for twenty-seven hours; and that when it did inform them, no mention was made of the release of TCDD.

ICMESA produced 130 tons of trichlorophenol in 1975 and 1976; a sizeable amount, according to the Italian commission. Roche and Givaudan will contend in their report that compared to the output of companies in the US and Germany this is a small amount. The commission's suggestion that the trichlorophenol reactor was poorly designed, is refuted by Givaudan, who claim that their design incorporated all the features necessary at the time for safe reactor operation.

The ICMESA reactor did not incorporate a dump tank to collect unwanted discharges from the reactor vessel. Givaudan thought this unnecessary, in view of the plant's other safety features. The commission, however, thinks this was a serious error.

A safety disc on the vessel was designed to rupture at the pre-set pressure of 3.5 atmospheres, and so discharge TCDD over Séveso. The Italian report states that a lower value would have caused a rupture at a less advanced stage of the reaction, with less disastrous consequences. Givaudan argue that the disc was incorporated to cope with mishaps at the beginning of the trichlorophenol reaction process—the accident at Séveso happened some six hours after the reaction was completed—and that the rupture pressure of the disc was selected for sound chemical reasons.

Central to the concern about the health of Séveso's inhabitants is the two week delay in their evacuation from TCDD contaminated areas. The commission says that Roche and Givaudan did not act swiftly enough to determine the extent of contamination. The companies contend that after a map of the polluted area had been made—a task which took two weeks—the proper authorities were informed and recommended by Roche to evacuate the area. Roche says that the authorities resisted initially until they appreciated the gravity of the situation.

Many scientists think the Séveso residents were exposed to low levels of TCDD. The commission disagrees, and recommends further studies to assess the long-term effects to health. TCDD is a potential carcinogen, and numerous scientists consider such studies essential. The International Agency for Research on Cancer (IARC), which has recently published a new report on the polychlorinated dibenzodioxins is currently assessing the chemicals' carcinogenicity. It is reviewing evidence from a number of the fourteen accidents which have occurred in trichlorophenol plants since 1949.

Alastair Hay

Venezuela: still out in the cold

IF Latin American research centres were graded by location, the greatest number of stars would surely be awarded to the Venezuelan Institute for Scientific Investigation (IVIC). Set on a mountain-top retreat 20 miles outside Caracas, its lush forest surroundings complemented by imposing works of modern sculpture, IVIC has all the environmental requirements of the archetypal ivory tower.

Yet its very isolation reflects a major complaint of Venezuelan scientists. Despite concerted efforts over a period of years to link scientific research to the country's technological needs, their activities remain marginal to the country's main economic and industrial activities.

IVIC itself, through a combination of generous private and public funding, has become, since its foundation in 1959, one of the leading basic research centres in Latin America. Its facilities in fields such as biophysics and analytical chemistry attract scientists from all over the continent. They often rival those in the laboratories of industrialised countries in which many of IVIC's research workers received their initial training.

The institute has also been actively developing its own postgraduate courses. There is a proposal before the next meeting of the United Nations Educational, Scientific and Cultural Organisation (UNESCO) in Paris, for example, that IVIC should become an information centre for postgraduate studies for the whole Latin American region.

Despite its scientific successes, however, the institute and its activities remain highly vulnerable to the effects of the country's changing fortunes, according to its director, Dr Luis M.

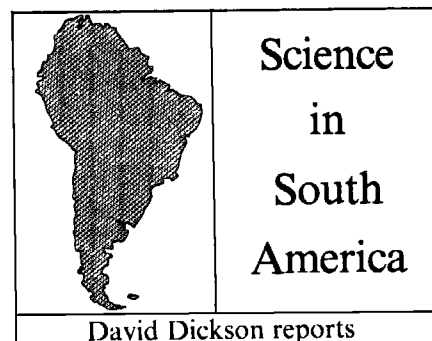
Carbonell. Earlier this year, for example, a 7% across-the-board cut in public expenditure—a response to a growing balance of payments problem and escalating inflation—resulted among other things in a six-month moratorium on travel to international conferences.

IVIC's vulnerability is itself a reflection of the extent to which research in Venezuela remains marginal to the country's main technological activities. A policy of pursuing rapid industrialisation while oil revenues continue to come in has largely been carried out through the transfer of technology from abroad; and although research and development expenditure, judged on a per capita basis, is among the highest in Latin America, it is still only 0.28% of the gross national product, less than a tenth of the proportion spent by many of the industrialised nations.

An attempt to rationalise the country's scientific efforts and link them more directly with its technological needs was made ten years ago with the setting up as an offspring of IVIC the National Council for Scientific and Technological Investigation (CONICIT), one of a number of similar planning institutions which have been established in various countries in Latin America.

"The marginality of science in Venezuela has largely been the result of a lack of communication between the scientific and the productive systems. Since its creation, CONICIT has been taking action to confront this situation," according to the council's president Dr Pedro Obregon, a widely-respected plant geneticist.

One area in which the council has enjoyed considerable success has been



in increasing the skills of those responsible for purchasing foreign technology in choosing that which is most appropriate to the country's needs; centres have already been established in London and Vienna to assist in this purpose and another will shortly be opened in Washington.

CONICIT has also been successful in stimulating new research initiatives in fields such as health and agriculture. In the latter case, for example, it recently opened a Centre for Agricultural Technology. Yet despite concerted attempts, CONICIT has so far had little success in generating the interests from the direction that, at least as far as the country's economy is concerned, matters most, namely sectors such as the chemical and steel industry.

Such industries, often under the government's rapid industrialisation policy and its support of joint ventures between national and foreign capital, have preferred to import technologies from abroad, rather than building up an indigenous research and development infrastructure. "The problem has been getting local industry interested in local technology" as one observer puts it; and the result is that CONICIT has been relatively unsuccessful in achieving one of its prime goals, namely increasing the country's technological



Institute for Scientific Investigation: five-star location but vulnerable to budget cuts and inflation, says director Luis Carbonell (above)

independence from foreign interests.

"Apart from opening the minds of many people to the problem of relating science to technical needs, and providing funds for training, I don't think CONICIT has been able to do much more in achieving its original goals," says Dr Carbonell of, IVIC who points out that 95% of patents taken out in Venezuela are still foreign, and that of the remaining nationally-owned patents, hardly any have been exploited.

CONICIT's problems are reflected at IVIC itself. One of the effects of the marginalisation of science is, it is claimed, a lack of respect for basic science as a profession; salaries are considerably lower than in universities, where teaching loads are so heavy that relatively little research is carried out.

In recent years IVIC has, for both ideological and financial reasons, been attempting to shift its focus away from a primary concern for basic science to embrace more applied subjects as well. The institute now has a small but active programme in solar energy research, concentrating in particular on methods of growing cadmium crystals, and is planning an investigation into techniques for the gasification of coal.

The institute also has a small research reactor, used not only for train-

ing engineers in handling nuclear materials but also for producing isotopes for medical and industrial use. At present, Venezuela has no plans for a nuclear energy programme; "but we are training people to make the right decisions, when these become necessary," says Dr Carbonell.

So far, however, as with CONICIT, industry's response to IVIC's efforts in the field of applied science has been disappointing. "Although lip service is given to the need to develop applied research, there does not really seem to be much real demand for it," according to Dr Marcel Roche, IVIC's first director and now editor of the Latin American journal *Interciencia*.

"Venezuelan industry tends to go for the maximum benefits with the lowest risks. The result is that research remains peripheral to the interests of industry; and this is a situation which has not been changed by CONICIT."

One attempt to improve things has been the drafting of a new law, similar to that which was introduced in Peru in 1971 (see below), which would oblige all industrial enterprises, to contribute a proportion of their gross profits to a research and development fund administered by CONICIT.

"In the future, it is essential that

research receives financial backing from the industrial sector. Not only will this provide research institutions with more resources, but it will also help protect industry from foreign interests," says Dr Obregon.

If the proposed law is accepted by the Venezuelan Congress—and this will depend largely on the outcome of the forthcoming presidential elections, as well as new Parliamentary elections next year—the result will be to put CONICIT in a much stronger position not merely to plan, but also to carry out a coordinated research strategy. It could also increase its budget, currently less than \$2m a year, by a factor of up to ten.

And what about the future of basic science? Dr Roche, a firm supporter of the need for developing countries to build a basic research capability to encourage technological independence, is optimistic.

"In my lifetime I have had the opportunity to see Venezuelan science grow at a relatively impressive rate. When I began to do research, there were in the country only six or seven professional investigators; now there are hundreds, well prepared and competent." □

Peru rings the changes in science policy

PERU has become the setting for a fierce debate on the respective roles that scientists and government planners should play in the design and administration of a national science policy. The focus of the debate is a proposal now before Peru's military leaders to upgrade the country's National Research Council, at present a primarily advisory body made up of eminent scientists, and to use it as the basis for establishing a "national system for science and technology".

The proposal is being resisted by groups such as the Institute for the Research in Industrial Technology (ITINTEC), which claim that a strongly centralised research policy would restrict the advantages of flexibility gained from the present system of relatively autonomous research institutes, each linked to separate government departments.

Others, including members of Peru's National Planning Institute—a government agency whose director has ministerial status—while agreeing on the need for a central programme for science and technology, argue that it should be conceived within, rather than parallel to, the existing social and economic planning machinery.

The root of the current conflict can be traced back to an ambiguity that

has existed since Peru's military government came to power in 1968. Keen to restrict the power of foreign interests and promote the country's technological independence, the government has encouraged the growth of a network of sectoral research institutes. There are now about 230 separate research institutes in Peru, half in the private and half in the public sector. And many, such as the Instituto del Mar del Peru (IMARPE) in Callao, enjoy an international reputation. At the same time, however, the government created as one of its first post-revolutionary acts the National Research Council, which theoretically has responsibility for establishing a national science and research policy.

"The problem is that when the government set up the council, it did not provide it with the authority or the resources to carry out the responsibilities it had been given," according to the council's current president, Dr Antonia Pinilla. "In consequence, the council has not been able to play the leadership role that it should have done."

As a remedy, Dr Pinilla, a previous Minister of Labour and a co-founder of the private University of Lima, has proposed a "national system" for co-ordinating the country's research

efforts. (Such a system has already been promised by the government in its Tupac Amaru Plan for returning to civilian rule by 1980).

The system would have two main components. The first would be a National Council for Science and Technology, a "normative body" responsible for setting policy goals and research priorities. The council would be made up of eminent scientists and those responsible for the various scientific and technological research institutes. The second would be an administrative body known as the National Institute for Science and Technology. In addition to carrying out the policies determined by the council, the institute would be responsible for setting up both an Advanced Centre for Science and Technology—a type of "Peruvian MIT", as Dr Pinilla describes it, concerned with postgraduate studies and research—and a national Centre for Scientific and Technological Documentation.

Dr Pinilla has suggested that the head of the national system act as both president of the council and as director of the institute, and that in recognition of the central role of science and technology in the country's economy, he or she be given equivalent ministerial status to the director of the

National Planning Institute.

The proposed science policy machinery has received enthusiastic support from amongst others, the science policy secretariat of the United Nations Educational, Scientific and Cultural Organisation (UNESCO), which has been promoting the "minister of science" approach for a number of years.

Others inside the country, however, are less enthusiastic. Despite Dr Pinilla's promise that the system will "respect the academic autonomy" of individual research institutions, they are worried that attempts will be made to impose unrealistic goals and objectives while limiting flexibility.

Particularly concerned are members of ITINTEC, an agency set up by the government in 1971 to stimulate research in the various areas of productive industry. At present, although working to support areas that have been given priority by the government, ITINTEC is able to operate relatively independently because its main income comes directly from industry.

Under the law establishing ITINTEC, all companies in Peru have to set aside 2% of their gross profits each year for research. A company can either submit for ITINTEC's approval a research project which it wishes to carry out itself, or hand the money over directly to ITINTEC, which will use it to finance its own research.

The model seems to have worked quite well, and the Venezuelan government is contemplating introducing a similar system (see above). Over a third of ITINTEC's research projects are carried out by universities, and the institute has established centres for wood technology and non-metallic materials, as well as what is claimed to be the best technical information service in Peru.

Furthermore ITINTEC has recently received a \$2 million grant from the US Agency for International Development to investigate the application of "appropriate technology" ideas to Peru—a project which will include studying the possibility of restoring the famous agricultural terraces built by the Incas, now realised to be a highly efficient means of using soil and rain-fall in mountain areas.

Under the proposed new system, although ITINTEC and other research institutes would remain responsible for developing and carrying out their own research programmes, they would be required to present plans and budgets to the National Council for prior approval. "We feel strongly that technological research and development should be carried out within each sector of industry, without being subject to any strong centralised control, which we have been lucky enough to avoid in the past," according to Dr



Inca terracing: old but efficient

Jorge Boggio, ITINTEC's director of technology. "Also, although we agree on the need to coordinate the country's scientific and technological research activities, I think it is wrong to give responsibility to a body whose members have had little experience of technological development.

Related views are expressed at the National Planning Institute, which has had a strong influence on carving out Peru's highly nationalistic industrial policy in recent years. "I feel that our country needs a very clear and long-term plan for science and technology, but it must be built on the long-term objectives of socio-economic planning," says the director of the institute's office of research and planning, Dr Fernando Gonzales Vigil. "We need to determine broad priorities first, and then inside these priorities to put an emphasis on specific areas of knowledge, rather than the other way round."

Much will depend on the attitude of the new Minister for Industry, Admiral Jorge Du Bois Gervase, a former head of the industrial service of Peru's navy and therefore, it is claimed, well aware of the problems of relating research to operational needs.

Another factor will be the effects of the austerity measures that the government has been forced to take to avoid defaulting on its loans from the International Monetary Fund. Although a proposal to eliminate industry's required contribution to ITINTEC has been successfully headed off, for example, the institute remains vulnerable.

But whatever the outcome, many feel that the policy finally adopted is likely to be one that increases support for the private sector, and weakens the role of state planners. "This would be the more logical outcome, taking into account the various national and foreign interests," says Dr Vigil. □

Andean Pact propose research tax on 1st world

ALREADY bidding for a place on the agenda of next year's UNCSTD meeting in Vienna is a proposal that has been put forward jointly by a group of Latin American countries and the Economic Commission for Latin America (ECLA) to establish a new fund for supporting technological development ventures in the Third World.

The countries concerned—Bolivia, Colombia, Ecuador, Peru and Venezuela—are members of the Andean Pact, an economic federation formed in 1969 to harmonise and coordinate the economic and technological policies of its constituent states.

From the beginning, the Andean Pact countries have expressed, mainly through the reports and studies of its secretariat's technical group, the Junta del Acuerdo de Cartagena, fierce criticism of the way that existing technology transfer and patent licensing policies have increased the dependency of the developing on the developed world.

The proposed fund is intended to strengthen the indigenous research and development capacities of developing countries. Contributions would be based on the size of the balance of trade on manufactured goods between rich and poor countries, with the general assumption that the greater the imbalance, the greater will be the technological gap.

Details of the proposals are now being revised in the light of comments made when the idea was discussed at the UNCSTD regional meeting for Latin America in Panama in August. Although doubts were raised by some of the countries present—mostly those claiming to be at an "intermediate" stage of development—others gave it enthusiastic support.

The Pact's position on technology, spelt out in a joint Decision 84 signed in June 1974, is based on the premise that if a country is to have control of its own development programmes, it is not enough merely to control the investment of capital (for example by regulating the financial activities of multinational companies). Equally important is control over the development and use of technology.

"It is easy to show, for example by looking at the way that the patent system has been used, that unless you have people who can control the



Cooperation to improve production technique. Left: coppermine in Chile. Right: ore analysis in Bolivia.

technology—and not just capital alone—you will remain in a position of dependence on the industrialised nations,” says Dr Luis Soto-Krebs, a Chilean metallurgist who is director of the Junta’s science and technology policy group.

One paragraph of Decision 84 states that “the high fixed costs of research and development necessitate careful choice of research priorities and the avoidance of duplication of effort”. The member countries agreed to develop sub-regional methods for generating new technologies through the application of local knowledge and research, and also to apply these techniques to some pilot projects.

One of the first projects was to improve techniques for copper production, a major source of revenue for Peru, Bolivia, and Chile (which was until 1975 a member of the Andean Pact).

Two problems were tackled simultaneously. The first was to develop new hydrometallurgical techniques for extracting copper from low grade ores; the second was to build up, virtually from scratch in the case of Bolivia, indigenous research and development that would cut down dependence on foreign technology and know-how.

Engineers and scientists from Peru and Bolivia went to Chile—the most advanced of the three in copper technology—for a one-month seminar in 1974 on the theory and laboratory-practice of copper extraction. They also spent some time working in different types of extraction plants in Chile. “The basic idea was that everyone in the project should have a common language; that people in the lab should know about the problems of the plant, and vice versa,” said Dr Soto-Krebs.

Members of the Bolivian team, which concentrated on methods of using conventional sulphuric acid leaching of the oxide ores, set to work on the design of a plant planned for the Corocora area by the Bolivian Mining Corporation (COMIBOL) that would process between 300 and 600 tons of ore a day.

Much of the work involved basic geological studies of the site of the

plant, and of the properties of the ores—often using testing facilities made available by Peru. Three years’ work resulted in detailed plans for what is proudly called “the first Bolivian-designed copper extraction plant” being handed over at the Junta’s headquarters in Lima earlier this year.

Officials hope that capital for building the plant will soon be made available, probably by West Germany, which helped to subsidise the initial research and development work. They also point out that the team which drew up the plans now has the experience to tackle other similar projects.

The Peruvians carried out much of their project in a research laboratory set up in La Oroya, the centre of Peru’s mining industry. The aim was to investigate ways of exploiting bacteriological leaching techniques for concentrating the ore, already known about by larger copper companies in the US, but little used or understood.

With the help of a German scientist who had been studying the relationship between bacteria and crystal structures, and two Canadian scientists who had been looking at applications of bacteriological leaching, Peruvian biochemists were able to separate out and reproduce those bacteria most suitable to local ores. Starting from ore samples no larger than 300 grams, the research built up until it was able to build and operate a pilot plant. “It soon became clear that we were almost in the front line of this research, and we came out with a method for very fast leaching very different from what was already being done,” says Dr Soto-Krebs.

The pilot plant is now producing two tons of copper a day at a considerably lower cost than conventional extraction techniques. Bacterial leaching also has the advantage of being non-polluting—since there are no exhaust gases to discharge into the air—and of being applicable to much lower grade ores than could previously be handled.

Since the success of the two copper projects, two other technological development projects have been set up by the Junta along similar cooperative

lines, one studying the use of forest products, a second looking at nutrition and food production. Other projects in the pipeline include a proposal for a joint investigation of techniques for the gasification of coal, of which Venezuela and Colombia in particular have large deposits.

“Too often our people are convinced they can do nothing . . . but we have now convinced people at the highest level that carrying out our own technological development is not a dream, and that investing in the generation of such technology is not useless,” says Dr Soto-Krebs.

Financing for similar types of projects could, it is claimed, be provided by the new fund which is being suggested to UNCTSD. The idea for such a fund was first raised at a meeting in Caracas earlier this year by Dr Francisco Sagasti, the Peruvian co-ordinator of a five-year study of science and technology policy in Latin America that has been financed by Canada’s IRDC.

“One area of top priority in stimulating cooperative research ventures between developing countries is providing financial support,” says Dr Sagasti. “What is being suggested is that the developed countries make available a sum proportional to the imbalance in trade in manufactured goods with the developing nations, calculated on a regional basis.”

Dr Sagasti estimates that, using 1977 trade figures and calculating on the basis of 0.25 per cent of the trade imbalance in manufactured goods, Latin American countries would receive about 60 million from the USA, Japan and members of the EEC alone.

“Such a mechanism would help to increase significantly the flow of funds for scientific and technological activities in the Third World, thus initiating a gradual process of redistribution of the world science and technological effort,” said Dr Sagasti. □

NEXT WEEK : a report by David Dickson on the role of the scientists in Brazil in criticising Brazilian energy policy; and environmental research in the Amazon

correspondence

Fusion first for USSR

SIR,—A short report was published (*Nature* 269, 370; 1977) concerning the "generation of neutrons by thermonuclear fusion using a concentric explosion with an exceptionally high degree of symmetry". The results were obtained by the Polish group headed by Professor S. Kaliski and were announced at an international conference in Prague.

Similar results obtained in the Soviet Union were made public in 1958 by L. A. Artsymovich at the Second International Conference of the Peaceful Uses of Atomic Energy held in Geneva. (L. A. Artsymovich, *Controlled fusion research in the USSR; Proceedings of the Second United Nations International Conference of the Peaceful Uses of Atomic Energy*, 2298, 31, 1958).

As early as 1955, 10^8 neutrons per impulse were generated in our experiments (which were mentioned by L. A. Artsymovich) by focusing converging spherical shock waves on a UD_2 target. In 1963 for UD_3 and gaseous D_2 targets this number was increased to 3×10^{11} . The converging shock wave was formed by implosion of a spherical charge of explosive. In most of the experiments the external diameter of the design was about 70 cm, the mass of gaseous D_2 being about 3×10^{-4} g.

Yours faithfully,
A. S. KOZYREV
V. A. ALEKSANDROV
N. A. POPOV

Leningrad, USSR

Correspondence and papers of Michael Polanyi

SIR,—A major part of Michael Polanyi's papers is now in the Joseph Regenstein Library at the University of Chicago. These are the papers that were in his possession in Oxford, but no systematic effort has been made so far to collect correspondence, manuscripts and memorabilia from people with whom he was in touch. The question as to how to collect this material comes at a time when a biography is being undertaken by Professor W. T. Scott of the University of Nevada in Reno. In the effort to combine the advantages of having original documents available for scholarly use at a centre in Britain, with access to the material by the biographer, and ultimate deposit of copies in the Chicago archive, we wish to make the following request.

We ask anyone possessing such material to make copies, and to send the originals to Miss Cicely Argyle, 12 Sunderland Avenue, Oxford. She will acknowledge receipt, and will deliver the material to the Contemporary Scientific Archives Centre in Oxford. This centre, under the direction of Professor Margaret Gowing, will process and catalogue the material and deliver it for permanent deposit to the Bodleian Library.

The making of copies before entrusting documents to the post is clearly important.

The biographer would appreciate having these copies sent to him, and undertakes to see that when he has made use of them they will finally be sent to the archive in Chicago to fill out that collection. He would also appreciate receiving any recollections and memorabilia, as well as suggestions of other sources of material. His address is: Professor W. T. Scott, Dept. of Physics, University of Nevada, Reno, Nevada 89557 U.S.A.

Yours faithfully,
G. N. BURKHARDT
W. MANSFIELD COOPER
ALISTER HARDY
ROBIN HODGKIN
ARTHUR KOESTLER
DRUSILLA SCOTT
TODD
T. F. TORRANCE
VERONICA WEDGWOOD

Effective trypanocides

SIR,—I was astonished to read the statement by your contributor Hugo Van den Bossche in his article 'Chemotherapy of parasitic infections', (22 June, page 626) that Berenil "is at present the only effective trypanocide left for use in cattle".

The reference given for this information is 'Williamson J., *Trop. Dis. Bull.* 73, 531–542 (1976)'. It is most unfortunate that an erroneous statement such as this should be perpetuated and re-published.

The facts are that several other products are available for the control of bovine trypanosomiasis, including 'Samorin' and 'Novidium' (May & Baker Ltd.), 'Ethidium' and 'Prothidium' (The Boots Co. Ltd.), 'Trypamidium' (S.P.E.C.I.A.) and 'Antrycide' (I.C.I. Ltd.).

Yours faithfully,
J. P. CAVILL

May & Baker Ltd,
Upminster, Essex, UK

Credibility of the anti-nuclear lobby

SIR,—I hope I am not too late to comment on Professor Pearce's thought-provoking contribution to the nuclear debate (20th July, p260).

●A "selective, admonishing and often haughty style" is surely more characteristic of the "anti-nuclear lobby" than of Mr Justice Parker.

●Difficult though Professor Pearce finds it to see how "any sane mortal would perpetrate false claims so as to foist an unsafe industry on an unsuspecting public", this appears to be exactly the opinion of many of the nuclear industry's critics. I also rather suspect that his remark about "the desire to be right and toe the party line" again describes an attitude more characteristic of the critics than of the industry.

●I doubt if anyone would argue that the nuclear power industry never makes

mistakes. But I would like to advance the apparently outlandish idea that the anti-nuclear lobby can make mistakes too.

●Professor Pearce's point about a possible change in the make-up of society invalidating the case for nuclear power is weak on two levels. First, what would we think of an administration which declined to build public housing, schools or hospitals, or even to reform itself, on the grounds that presently prevailing values cannot be assumed to be those of the future? Second, even if society changes world-wide in the direction the critics wish, there will still be a demand (albeit reduced) for central power stations. Is there not then a case for building nuclear plant, and allowing the dearer, dirtier and more dangerous coal and oil burners to be phased out? To forestall a possible objection, I fully agree that a community limited to near the surface of one planet cannot continue to grow indefinitely. But why the optimum level of economic activity should be just that of the developed world in 1978 has never been explained, to my satisfaction at least.

●It is certainly proper to put a case against nuclear power. But the advocates of this case are surely honour bound (as I think Professor Pearce would agree) both to examine this case themselves, and to permit others to do so. It is my distinct impression that many of the present 'antis' do neither now, and would do neither in the future in Professor Pearce's "institutions to debate and evaluate those alternatives".

●While not all the opposition to nuclear power is irrational, a great deal of it certainly is, and it is surely unfair to castigate as "hysterical outbursts" any statement to this effect.

●In the face of some of the arguments put to him, is it not understandable that Mr Justice Parker should occasionally be "perplexed and confused"? For example, reportedly one suggestion was that future energy needs should be met with "cosmic forces". This sort of talk perplexes and confuses even me, and if that makes me a haughty and unfeeling rationalist, so be it.

●Whether the debate becomes a conflict is surely in the hands of the critics as much as of the industry. The death of a demonstrator at Creys Malville in France makes this point sadly dramatic. A recent article in *New Scientist* (3 August, p349) is distinctly ominous, and I for one would welcome Professor Pearce's comments on the kind of tactics hinted at therein.

Lastly because of the tone of the "debate", I had better state that I am a physicist with no professional connection to the nuclear industry. I also hold that complete lack of bias, like perfect social justice, is an unattainable ideal. But, again like social justice, it is very much worth-while to work towards it.

Yours faithfully,
J. F. CRAWFORD

Klein Dottingen
Switzerland

news and views

GABA and benzodiazepine receptors

from Leslie L. Iversen

MINOR tranquillisers of the benzodiazepine series (diazepam = Valium for example) are the most widely prescribed of all psychotropic drugs. The discovery last year (Squires & Braestrup *Nature* **255**, 732; 1977; Möhler & Okada *Science* **198**, 849; 1977; see *News and Views* **266**, 678; 1977) of specific high affinity binding sites for ^3H -diazepam in mammalian brain, possibly representing a novel type of 'benzodiazepine receptor', was thus an exciting breakthrough.

Previous work on this group of drugs had suggested that they might mimic or enhance the synaptic actions of the inhibitory amino acid transmitter γ -aminobutyric acid (GABA) in the brain. At first, biochemical studies failed to reveal any direct connection between benzodiazepines and GABA at the receptor level. Thus benzodiazepines did not influence the binding of ^3H -GABA to its receptor sites in brain membrane preparations, and conversely GABA and related drugs did not influence ^3H -diazepam binding. A series of recent studies, however, now suggests that some interaction may exist between the two types of receptor. Thus, Tallman *et al.* (*Nature* **274**, 383; 1978) report that GABA and the GABA-like drug muscimol cause an increase in the apparent affinity of binding sites for ^3H -diazepam in rat cortical membranes *in vitro*. This effect is blocked by the GABA antagonist bicuculline, which itself lowers the affinity of the drug receptor sites for ^3H -diazepam.

Other studies suggest that, as with enkephalins and opiate receptors, brain normally contains an endogenous ligand for the benzodiazepine sites. Thus, Karobath *et al.* (*Eur. J. Pharmacol.* **49**, 323; 1978) and Marangos *et al.* (*Life Sci.* **22**, 1893; 1978) describe the existence in acid acetone extracts of bovine brain of substance(s) (molecular weight less than 1000) which inhibit the binding of ^3H -diazepam. The specificity of these compounds, however, is not yet clear. The substance described by Karobath *et al.* for example, was present not only in brain but could be detected in even larger amounts in peripheral tissues such as heart and skeletal muscle.

A very interesting proposal has been made by Toffano *et al.* (*Proc. natn.*

Acad. Sci. U.S.A., **75**, 4024; 1978), (see also Guidotti *et al.* *Alfred Benzon Foundation Symposium GABA Neurotransmitters*, 22–25 May, 1978, Munksgaard, Copenhagen, in press; Guidotti *et al.* this issue of *Nature*, page 535), who describe the purification from rat brain of a protein which seems to act both as a ligand for benzodiazepine receptors and as a modulator of GABA receptors. They studied the binding of ^3H -GABA to synaptic membranes from rat brain *in vitro*, and confirmed what others have found (Enna & Snyder *Molec. Pharmacol.* **13**, 442; 1977; Greenlee *et al.* *Life Sci.* **22**, 1653; 1978), namely that high affinity binding of ^3H -GABA is not detectable in freshly prepared membranes but becomes evident only after the membranes have been subjected to exhaustive washing. In fresh membrane preparations only a limited amount of specific ^3H -GABA binding, with relatively low affinity ($K_d \sim 200$ nM) can be detected, whereas after repeated washing, freezing and detergent treatment specific binding increases and a high affinity component ($K_d \sim 20$ nM) is revealed. The supernatant fractions from fresh membrane preparations contained a substance which inhibited high affinity GABA binding when added to washed membranes. Chromatographic purification of this material yielded a 500-fold enrichment of a heat stable protein, with apparent molecular weight 15,000. The possible relation of this endogenous inhibitor of GABA receptors to benzodiazepine receptors stems from the observation that the purified substance is able to inhibit the binding of ^3H -diazepam when brain membranes are preincubated in its presence. Furthermore, incubation of fresh membrane preparations with quite low concentrations of various pharmacologically active benzodiazepines seems to displace the endogenous inhibitor and converts the GABA binding properties of such membranes

to the high affinity type normally seen after removal of the inhibitor protein by washing. Thus, the benzodiazepines might act normally to enhance the actions of GABA at its receptors by displacing an endogenous modulator protein which forms part of a macromolecular complex constituting the GABA receptor-ionophore.

This attractive hypothesis will certainly generate a great deal of interest and further work. It is perhaps surprising that small amounts of the endogenous GABA receptor inhibitor could be detected in non-cerebral tissues such as liver. The relevance of this mechanism to the pharmacological actions of benzodiazepines also rests critically on the correlation between the known pharmacological actions of various benzodiazepines *in vivo* and their ability to displace the endogenous modulator protein from brain membranes; so far only a limited number of such drugs have been tested.

Our understanding of the cellular localisation and molecular nature of the benzodiazepine receptors remains limited. Labelling of benzodiazepine receptors *in vivo* has been demonstrated using ^3H -flunitrazepam (Chang & Snyder *Eur. J. Pharmacol.* **48**, 213; 1978) and ^3H -diazepam (Willamson *et al.* page 533, this issue of *Nature*). The findings of Chang *et al.* (*Proc. natn. Acad. Sci. U.S.A.* in the press) suggest that these receptors are located in large part on glial cells in rat brain. They report that a variety of chemical or surgical lesions which destroy the neuronal elements in corpus striatum or cerebellum of rat brain fail to alter the density of benzodiazepine binding sites in these brain areas. Furthermore, benzodiazepine sites were enriched in subcellular fractions containing glial cells, and like Guidotti *et al.*, Chang *et al.* were able to detect high affinity benzodiazepine binding sites in membranes prepared from rat C6 glioma cells. The postulated glial localisation of benzodiazepine sites, however, is not easy to reconcile with the notion that these sites interact directly or indirectly with GABA receptors, which are thought to be located largely on neurones. The fact that ^3H -diazepam and ^3H -GABA binding sites do not show parallel distributions among brain regions in any case suggests that they are not necessarily linked—and indeed may not even occur on the same cellular elements. □

Leslie L. Iversen is Director of the MRC Unit of Neurochemical Pharmacology, Cambridge.

Anomalous cosmochronology . . .

from Stephen Moorbath and David N. Schramm

FROM the comprehensive sweep of topics at a recent conference on the use of isotopic dating in geology and cosmology*, it is clear that isotopes can shed light on almost every aspect of Earth and Planetary Sciences. Reviews ranging from their use in dating the Universe to their use in deducing the diet of our ancestors indeed provided food for thought.

There has been a revolution in our understanding of the early Solar System as a result of the discoveries of isotopic anomalies in Allende and other carbonaceous chondritic meteorites. The discoveries were led off in 1973 by Clayton and his group at the University of Chicago, who discovered that the oxygen isotopic composition had large variations which could not be explained by simple fractionation and seemed to require an admixture of a different nucleosynthetic component than that of the bulk of the Solar System material. The addition of these non-standard nucleosynthetic components was given a timescale when Typhoon Lee, D. Papanastassiou, and G. J. Wasserburg (Caltech) showed that ^{26}Al was present when objects in the Solar System solidified. Since ^{26}Al was presumably made in a supernova and ^{26}Al has a half-life of only 7×10^5 yr, this seemed to indicate that a supernova explosion had occurred within a few million years of the formation of the Solar System, and had injected freshly synthesised material into the protosolar cloud. With these observations in mind, various astrophysical theorists have developed models for forming the Solar System utilising supernova shocks and grain formation and penetration as a means of getting the anomalies from the supernova into the protosolar cloud.

New isotopic anomalies

At the Snowmass conference, observations of new isotopic anomalies both elucidated and complicated the scenario. Although most of the rocks in Allende have almost normal Solar System isotopic composition, with the exception of oxygen anomalies and excess ^{26}Mg due to the decay of ^{26}Al , it was found about a year ago that two inclusions in Allende, labelled C-1 and EK 1-4-1, were anomalous for almost every element included. In addition, the nature of the isotopic anomalies was different in each inclusion. For ex-

ample, where one particular set of neutron-rich isotopes might be enriched in EK 1-4-1, the same set is depleted in C-1. The discovery of the anomalies in these samples was made by Clayton's group, Wasserburg's group, and G. Lugmair (University of California, San Diego). New and extremely intriguing isotopic information on these two inclusions was presented. In particular, hopes for a simple explanation tying all the anomalies to one type of nucleosynthetic process were complicated by the discovery that in some mineral separates both proton-rich and neutron-rich anomalies were found, whereas in others only one or the other was present. Also, whereas C-1 seems to show more elements anomalous than normal material, it is depleted in the amount of freshly injected ^{26}Al . Since the anomalies in C-1 for the most part tend to be depletions rather than additions, this may mean that C-1 is a 'truer' sample of the protosolar material, and what we now call normal is really a well-mixed sample, including the ejecta from a supernova. Perhaps EK 1-4-1 is a sample in which the supernova ejecta is enriched relative to normal. The question of the composition of the different components which went to make up the Solar System and eventually became mixed together to yield what we call normalised isotopic composition will continue to be worked on, and there are, no doubt, going to be many new surprises.

Universality

The universal nature of the ^{26}Al chronology, and thus its significance, was strengthened considerably by new results reported by J. C. Lorin and M. C. Michel-Levy (Paris) who showed that ^{26}Al anomalies are also present in the Leoville carbonaceous chondrite, whilst J. D. MacDougall (University of California, San Diego) and D. Phinney (Johnson Space Center) showed that the carbonaceous chondrite Murchison also had ^{26}Al enrichment comparable to that in Allende. They also found that some samples of Murchison showed extremely large mass fractionation of Mg isotopes. This large mass fractionation is intriguing because the only other samples that have shown large isotopic fractionation were EK 1-4-1 and C-1.

One point which received some attention was the relation between the 10^6 -yr ^{26}Al time scale and the 10^8 -yr time scale relating the last nucleosynthetic event to produce ^{129}I and ^{244}Pu to the time when xenon daughter products of these two radioactive nuclei

were retained in meteorites. Typhoon Lee and his collaborators at Chicago showed that it may be quite possible for a supernova simultaneously to make the ^{26}Al and the neutron-rich heavy anomalies observed in EK 1-4-1 and yet not produce ^{129}I and ^{244}Pu . On the subject of ^{129}I , one point brought out by A. Zaikowski (University of California, Berkeley) was that in Allende there seem to be at least two separate periods of condensation separated by about 3.7 Myr. That is, the ^{129}I seemed to be included in some rocks 3.7 Myr before others. This shows that some material in a meteorite may be considerably more primitive than other material in that same meteorite. C. Allègre and a team at Paris are trying to utilise the different nucleochronologies and retention times from Pb, Sr, Ar and Xe to show that the time/temperature history of the early Solar System was probably not monotonic, but went through at least one secondary reheating stage for which he set some parameters, but not all.

Hints of possible new results which have not yet been substantiated, but which if true would be extremely exciting, were presented by O. Manuel (University of Missouri, Rolla) who showed that there may be a correlation of Te isotopic anomalies with the strange Xe anomalies found enhanced in some carbonaceous chondrite material by E. Anders and his group at Chicago. Another intriguing suggestion based on preliminary experimental results was that of J. L. McCrumb and his collaborators at San Diego, who suggested that it may be possible for chemical fractionation to produce non-linear isotopic effects. If true, this would have extremely important implications for interpretations of such things as the oxygen isotopic anomaly.

There was also discussion of cosmological timescales as well as the discussion of timescales in the early Solar System. It was pointed out by K. Hainebach and his collaborators that a consistent age for the Universe can be obtained, utilising not just nucleocosmochronologies, but also the traditional Hubble dynamics and globular cluster ages and big bang nucleosynthesis. The age for the Universe where all methods agree is between 13.5 and 15.5 billion yr.

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*The Fourth International Conference on Geochronology, Cosmochronology and Isotope Geology was held at Snowmass-at-Aspen, Colorado, August 20-25, 1978.

... and geochronological anomalies

MANY papers discussed the application of all the available age methods to igneous, metamorphic and sedimentary rocks from all over the world, ranging in age from ancient to almost modern. Finding the oldest terrestrial rocks is a fashionable and prestigious exercise. J. Barton Jr. (Witwatersrand University) obliged with gneisses from the Limpopo belt of southern Africa which yielded a rubidium-strontium whole rock isochron age of nearly 3.8 billion yr, although the uranium-lead systematics of these rocks are highly complex and gave younger ages. At the other end of the geological time scale, the controversy regarding the age of the KBS-tuff, an important marker horizon in the Plio-Pleistocene succession of Northern Kenya, continues unabated. G. H. Curtis and his collaborators (University of California) favour an age of ~ 1.8 Myr, whereas F. J. Fitch and coworkers, from the Universities of London and Cambridge, favour ~ 2.5 million years. Clearly a tuff nut to crack!

The new and exciting samarium-neodymium age method was thoroughly aired by many workers. The geochemical coherence of parent and daughter forms a sharp contrast to the rubidium-strontium and uranium-lead methods and offers the possibility of tracing crustal evolution in certain geological environments which could wholly or partly disturb rubidium-strontium and uranium-lead systematics.

Geochemistry

On the application of initial strontium, lead and neodymium isotopic ratios to petrogenetic studies of igneous rocks and of the geochemical and temporal evolution of the Earth's continental and oceanic crust, and of the upper mantle, most speakers considered that geochemical heterogeneities have existed in the upper mantle for at least 1 to 2 billion years, as judged from isotopic measurements on oceanic basalts. Another issue, still highly controversial, concerns the origin of calc-alkaline lavas (mostly andesites) at continental margins, such as the west coast of South America. Conflicting interpretations of 'anomalous' isotopic and trace-element compositions were proposed by D. E. James (Carnegie Institution, Washington) and by J. R. Lancelot and colleagues (University of Montpellier and United States Geological Survey, Denver). James favours derivation of andesites by massive subduction and partial melting of vast quantities of sediments of continental

origin, whereas Lancelot *et al.* prefer selective sialic contamination with radiogenic strontium of an essentially mantle-derived magma with low $^{87}\text{Sr}/^{86}\text{Sr}$ ratio during fractional crystallisation of the calc-alkaline liquid. This controversy is closely related to the problem of whether the continental crust grows through geological time, or whether its volume has remained essentially constant. I (S.M.) believe that the bulk of isotopic, geological, geochemical and geophysical evidence very definitely favours continental growth, but both arguments will continue for a long time yet. Nonetheless it is now certain that continental igneous rocks frequently acquire an isotopic imprint of the older continental crust through which they make their way to the surface, and this phenomenon can be used to provide constraints for the age and nature of rocks at depth which are not exposed at the surface at that particular locality. P. N. Taylor and S. Moorbath (University of Oxford) reported that the 3.7 billion yr-old sialic craton of West Greenland appears to underlie only a very small part of the voluminous, extensively exposed 2.9 billion yr-old craton. Furthermore, W. Compston and B. Chappell (Australian National Uni-

versity) believe that they have demonstrated that Precambrian continental material was present in the lower crust of southeastern Australia during the early Palaeozoic, so that crust in this region did not originate solely as new oceanic crust at that time.

On the non-radiogenic stable isotope front, the oxygen isotopes as always offer a wide range of fundamental geological applications. F. J. Longstaffe, H. P. Schwarcz and R. H. McNutt (McMaster University) have shown that up to the onset of partial melting the oxygen isotope ratios of Archaean granitoid gneisses reflect the nature of their protoliths. Fortunately, the conclusions closely parallel those drawn from radiogenic isotope studies. Furthermore, the irrepressible H. P. Taylor, Jr. (Caltech), as usual presenting as many new data as all other workers combined, showed how oxygen isotope ratios vary sympathetically with initial strontium isotope ratios in passing from west to east in the great Peninsular Ranges Batholith of Southern and Baja California, and how this must reflect an increasing crustal component in the petrogenesis of this major rock unit.

This was an important and magnificently organised conference. Those geologists who nowadays still ignore, misunderstand and disbelieve constraints imposed by isotopic and geochronological data very definitely do so at their own peril. $\square$

Presynaptic receptors

from S. Z. Langer

THE classical picture of events at nerve endings and synapses has been modified over the past few years by accumulating evidence for the existence of presynaptic receptors for neurotransmitters, in addition to the classical postsynaptic receptors which mediate the response of the effector organ (in the periphery) or of the postsynaptic neurone (in the central nervous system). The presynaptic receptors, found on the outer surface of the nerve endings from which neurotransmitter is released, are thought to be involved in the modulation of the neurotransmitter release evoked by stimulation, by means of a negative feedback mechanism in which a neurotransmitter regulates its own release.

As U. S. von Euler (Karolinska Institute, Stockholm) pointed out in his opening lecture at the First International Symposium on Presynaptic Receptors*, the first indications of the existence of presynaptic receptors can

be traced back as far as 1957 when results on the effects of alpha-adrenoceptor blocking drugs on the stimulation-evoked release of noradrenaline from noradrenergic nerve endings triggered considerable interest (Brown & Gillespie *J. Physiol. Lond.* **138**, 81; 1957). It was not until 1971, however, that the hypothesis was formulated that alpha-adrenoreceptors are present in noradrenergic nerve endings and are involved in negative feedback control of noradrenaline release. Since then evidence for presynaptic receptors in the peripheral and central nervous systems has continued to accumulate (see for reviews Langer *Biochem. Pharmacol.* **23**, 1793; 1974; *Br. J. Pharmacol.* **60**, 481; 1977; Starke *Rev. Physiol. Biochem. Pharmacol.* **77**, 1; 1977; Westfall *Physiol. Rev.* **57**, 659; 1977).

Evidence for the presynaptic location of the alpha-adrenoreceptors that regulate noradrenaline release has been found in the rat heart. In this tissue there is a significant reduction of ^3H -dihydroergocryptine binding after de-

*Held in Paris on 22-23 July, 1978.

generation of the noradrenergic nerve endings induced by the administration of 6-hydroxydopamine. The loss of alpha-adrenoceptor binding sites after chemical sympathectomy indicates that some of these sites are located presynaptically on noradrenergic nerve endings.

Several contributions stressed the fact that there are differences in affinities for agonists as well as for antagonists between the postsynaptic alpha-adrenoceptors that mediate the responses of the effector organ and the presynaptic inhibitory alpha-adrenoceptors that regulate the release of noradrenaline during nerve stimulation. These differences open up the possibility that selective presynaptic agonists or antagonists could be developed to elicit pharmacological actions as a result of effects on neurotransmission. These changes in transmitter release can be brought about through the selective activation or blockade of presynaptic receptors, and might be of therapeutic importance in conditions such as hypertension, depression and schizophrenia.

In addition to the presynaptic inhibitory alpha-adrenoceptors that regulate noradrenaline release in the peripheral and the central nervous systems, presynaptic receptors involved in negative feedback mechanisms for other neurotransmitters have been reported in the peripheral and in the central nervous systems. Evidence for presynaptic inhibitory muscarinic receptors was presented for peripheral as well as for central cholinergic nerve endings. The presence of presynaptic inhibitory dopamine receptors was reported in the rabbit nucleus caudatus and also in the rat striatum. In addition, results were presented which are compatible with the presence of presynaptic inhibitory GABA receptors in the GABA-ergic nerve endings of the rat substantia nigra. It is therefore possible that in addition to the presynaptic receptor systems involved in the autoregulation of the release of noradrenaline, acetylcholine, dopamine and GABA, that presynaptic receptors for other neurotransmitters might be present in nerve endings to subserve the function of the fine regulation of transmitter release upon the arrival of nerve impulses.

In addition to the presynaptic receptors through which the neurotransmitter can regulate its own release, a real mosaic of receptors seems to be present on the noradrenergic nerve endings. Evidence was presented for several presynaptic receptors which are involved in the inhibition of noradrenaline release during nerve stimulation: the muscarinic receptor, the dopamine receptor, the prostaglandins of the E series and in some neurones, the opiate

and adenosine presynaptic inhibitory receptors. Presynaptic receptors involved in the facilitation of the stimulation-evoked release of noradrenaline include beta-adrenoceptors and also angiotensin II receptors. These presynaptic receptors can be activated by other neurotransmitters (acetylcholine) by circulating endogenous compounds (adrenaline, angiotensin II) or to locally produced substances (prostaglandin, adenosine) to modulate transmitter release.

In the central nervous system there is increasing evidence for the modulation of the release of neurotransmitters through presynaptic receptor mechanisms. In the rat cerebral cortex β -endorphin and enkephalin reduce, while GABA enhances the stimulation-evoked release of noradrenaline. In

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addition, the stimulation-evoked release of dopamine from dopaminergic nerve endings can be modified by acetylcholine and GABA. Another example is the inhibition by enkephalin of the potassium-evoked release of substance P. It was concluded that local neurotransmitter interactions involving presynaptic receptor systems may represent a physiological mechanism for the modulation of transmitter release in the central nervous system. Attention was drawn to the fact that presynaptic receptors can be acted on by exogenously administered agonists or antagonists to modulate the release of neurotransmitters induced by nerve impulses. The potential therapeutic relevance of this principle was discussed and it seems that presynaptic receptors in the peripheral and the central nervous system could be considered as target receptors for the development of new drugs. □

Intraplate earthquakes

from Peter J. Smith

MOST of the world's earthquakes occur in narrow bands clearly associated with plate boundaries. To say that the causes and mechanisms of such earthquakes are therefore perfectly understood would be to exaggerate, but at least it is possible to see in general terms that large-scale plate boundary could quite easily lead to violent releases of stress. But what about intraplate earthquakes, of which there is a not insignificant number? By definition there are no intraplate active oceanic ridges, subduction zones or transform faults. So what causes earthquakes away from plate boundaries?

There are several possibilities. Some intraplate earthquakes may be associated with volcanism, and some specifically oceanic shocks registering on seismographs may be due to underwater slumping. Other seismic events seem to represent slippage of local faults. Whether such faults are always as local in cause as they are in geography is another matter, however, for some have probably been left over from the time when their present localities were closely associated with plate boundaries and others may result from stresses in the interior of a plate viewed as a membrane ('membrane tectonics')—stresses that are bound to be influenced by what happens at the edge of the plate.

All three of these causes ('slumping,

fracture zone activity, and possible submarine volcanism') have been invoked in a very general way by Estill and Odegard (*Geophys. Res. Lett.* **5**, 485; 1978) to account for seismicity in the vicinity of the Hawaiian Islands. Estill and Odegard were much less concerned with causes, however, than with the fact that the Hawaiian ridge appears to be more seismically active than previously thought. They report that between July 30, 1976 and March 30, 1977 the Makapuu Ocean Bottom Seismograph, which lies 10 km off the south-east coast of Oahu, recorded no fewer than 359 local events. Most of the epicentres determined (65 in all) occurred on, and in the immediate vicinity of, Hawaii, which is understandable, although two events of $M_L > 4$ took place near Oahu and Niihau, respectively, well away from the currently active volcanic centre.

But if Estill and Odegard have produced an observational surprise, Campbell (*Geophys. Res. Lett.* **5**, 477; 1978), who is more concerned with cause than with observation, has come up with a theoretical one. As part of a re-examination of the Charleston, South Carolina, earthquake of 1886 (*U.S. Geol. Survey Prof. Paper* 1028; 1977) Long and Champion recently showed that earthquakes in the Charleston area occur in, or very close to, a large mafic/ultramafic crustal intrusion, and Kane has pointed out that there are similar intrusions in several other seismic regions of eastern North America. This has led several people to suggest

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that some local earthquakes may be caused by the stress concentrations set up by such intrusions in the felsic continental plate.

Campbell has now treated this problem (or at least a simplified version of it) analytically. He shows that a vertical intrusion of circular or elliptical cross section is likely to produce stress concentrations only 20–30% greater than the regional stress if, as most workers have assumed, the intrusion has a greater rigidity ($\times 2$ maximum) than that of the rest of the crust. In general, such a stress differential is unlikely to give rise to local seismicity. But if, as Kane suggested, the intrusion has been serpentinised, the picture is transformed. A serpentinised intrusion could have a rigidity up to 10 times lower than that of the surrounding crust, which could lead to stress differentials of up to 200%. Under these circumstances it is quite possible that

earthquakes would occur in the continental plate close to the intrusion.

Of course, it is quite evident that not all crustal intrusions give rise to earthquakes. Campbell speculates that this may be because some intrusions do not have mechanical properties sufficiently different from those of their surroundings (that is, they are not serpentinised enough); because some do not go deep enough, do not have a small enough radius of curvature, and are not oriented appropriately to the regional stress field (as demanded by the model); and because some, though not excluded by these two considerations, may nevertheless simply be too small to produce other than very low intensity events. Crustal intrusions may not account for all, or even most, intraplate earthquakes, but as a possible cause they must certainly now be added to the existing list. □

Drosophila genetics at Szeged

from Michael Ashburner and Peter Lawrence

FOR peripatetic *Drosophila* geneticists the long hot summer ended with a meeting on *Drosophila* biology at Szeged in Hungary*. Less 'molecular' than the recent conferences in Crete (see *News & Views* 275, 364; 1978) it provided an opportunity to meet many workers from Communist countries. In view of the widespread boycott of the Moscow International Genetics Congress this summer, this

opportunity was especially welcome and opened many eyes to work going on in Eastern Europe and the academic centre, Akademgorodok, in Siberia.

Bands and interbands

The problem of the relationship between the 'genes' of *Drosophila* and the bands of the polytene chromosomes, and the problem of the genetic significance of bands and interbands, continues to be a challenge as we heard in a spirited paper from I. Zhimulev of the Soviet Academy of Sciences Institute of Cytology and

Genetics in Akademgorodok. Zhimulev and his collaborators have taken a very small region of the X chromosome of *Drosophila*, including the large band 10A1.2, long famous as the site of the eye colour mutant *vermilion*, and attempted to discover other genetic functions coded for by the same band. It would seem that this very large band does include the information for, in addition to *vermilion* (which codes for tryptophan pyrrolase), at least one and possibly three vital loci. All of these complementation groups seem, from deficiency mapping, to be located at the edges of the band, the bulk of whose material appears to be genetically silent.

Zhimulev is a proponent of the idea that bands do not represent structural or functional units but are, in effect, epigenetic phenomena reflecting only the state of gene activity within a tissue. In Zhimulev's view interbands are active genes, a theory suggested by others. This of course is anathema to those of us who thought that Beermann had dealt a death blow to the 'Istanbul hypothesis', for this would imply that the similarities in banding seen between the chromosomes of different tissues reflect only similarities in transcriptional activity, perhaps largely of what are called, not always with due humility, 'house-keeping' genes. To be certain there are other straws in the wind which indicate that perhaps a major re-evaluation of the problem may not be overdue: to mention but two: D. Ribbert (of the University of Munster) reported last year to the Helsinki Chromosome Conference that the banding patterns of the polytene chromosomes from two tissues of the fly *Calliphora* were completely different and now we have evidence from the gene cloners that 'identical' bands (for example in different stocks of *D. melanogaster* or in different, but related species) may differ in their base sequence, at least in part. Clearly the whole problem should be filed under 'keep a watching brief'.

Related to this subject are studies attempting to analyse genetically structures which everyone agrees are active genes—that is to say the puffs of the polytene chromosomes. Two such were discussed at Szeged—the 87A/87C 'heat shock' inducible puffs (J. Gausz and colleagues, Szeged) and the 2B early ecdysone inducible puff (E. S. Belyaeva & Zhimulev, Akademgorodok). This difficult approach to the study of gene control in *Drosophila* has yet to bear fruit but at

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THE Biological Research Centre of Szeged, known as Hot Spring Harbor on account of the hot, sulphurous, local waters, was founded some 6 years ago and is the showpiece of Hungarian biology. More than 100 workers are organised into four Institutes, housed in modern and well equipped buildings. An important feature of the Centre is that for 5 years it has benefited from a large UNESCO grant, under the United Nations Development Programme, that in addition to allowing it to purchase equipment has enabled sending many of their younger scientists abroad for periods of one or two years. For example the small *Drosophila* and nematode group, under Istvan Kiss, brought together

biologists from various different fields and nearly all have, in effect, been trained during stays in the laboratories of Western Europe and North America. In many cases the close collaboration between these Hungarian scientists and their former colleagues continues and many of the papers presented at the meeting attested to the value, to both sides, of these contacts. More novel has been the programme within the BRC to train scientists from Third World countries — by scholarships many students, who would otherwise often find it difficult to receive such a training, have been brought to Hungary for quite long periods to collaborate with the Hungarian scientists.

*A Workshop on Current Topics in Insect Development was held at Szeged, Hungary on 12–15 September and hosted by the Hungarian Academy of Sciences Research Centre, Szeged. It was supported by UNESCO and the International Cell Research Organisation.

2B several lethal complementation groups have been identified and some alleles seem to show abnormalities in the normal temporal sequence of gene activities inducible by ecdysone in salivary glands. Since the 2B puff is one of the first to respond to the hormone the implications of these observations for the analysis of cascade control reactions during development are clear to see.

Ecdysone receptors

Another topic of enduring interest is that of the general control of development by ecdysone. The meeting was fascinated by accounts of two temperature-sensitive ecdysone-deficient mutants by J. Lepesent (University of Paris) and J. Holden (Queen's University, Kingston), which will be invaluable for the study of ecdysone action. Even more fundamental, however, is the problem of the ecdysone receptors. So elusive have these been that some were beginning to doubt their very existence on anything other than theoretical grounds. The breakthrough by J. D. O'Connor (University of California, Los Angeles) and by Fristrom's group in Berkeley, has now been made. What was needed was a radiolabelled analogue of ecdysone of very high biological activity and very high specific activity. This was synthesised by the tritiated reduction of the phytoecdysone stakisterone C to the very active ponasterone A. Using tissue culture cells O'Connor and his group have characterised a proteinaceous binding activity that does everything an ecdysone receptor should do, at least to those acquainted with vertebrate steroid receptors. Thus the binding shows a temperature-dependent nuclear translocation, a translocation accompanied by a change in sedimentation constant, it is saturable and shows biologically realistic affinity constants to various ecdysone analogues. Moreover derivatives of the original cell line exist which are unresponsive to the hormone and at least some of these seem to lack the receptor. The receptor's purification and localisation to ecdysone-treated salivary gland chromosomes is eagerly anticipated. If current attempts to isolate the ecdysone-responsive genes on

plasmids succeed we are in for an exciting few years.

Sex determination

R. Nöthiger and his colleagues (University of Zurich) are throwing new light on an old problem, at least as far as *Drosophila* is concerned. For years *Drosophila* geneticists have accepted the balance theory of sex determination of Bridges, whereby sex is determined by the ratio between the number of X chromosomes and autosomes, without enquiring too deeply just what this may mean in genetic or developmental terms. Nöthiger suggests that, all organisms, whether man, beast, or green things, possess a 'key gene' whose activation or inactivation is required for sexual development along one or other pathway. In different organisms different methods will be used to control the activity of this key gene: in some (the marine worm *Bonellia*) the signal will be environmental, in others genetic. In *Drosophila* it would be this key gene that responds, by an unknown mechanism, to the X chromosome: autosome balance. The report of T. Cline of Princeton University of a new locus on the X chromosome, which appears to be normally 'on' in females and 'off' in males, and may thus correspond to Nöthiger's proposed key gene, is therefore particularly exciting. Cline has found that if the normal state of affairs is disturbed by mutation, sex-limited lethality results. Nöthiger's laboratory has been systematically analysing the several mutant loci known in *Drosophila* which effect a sex reversal and it was suggested that perhaps the activity of their wild-type alleles is normally subordinate to that of the X-linked key gene.

Cell division and death

The control of cell division and cell death in growing organs remains a mystery, but *Drosophila* geneticists are making a start on its analysis. It has been known for some time that when wild-type cells are generated in a slow growing (*Minute/Minute*⁺) background they grow fast and take over much of the compartment. Morata and Ripoll (*Devl. Biol.* 42, 211; 1975) discovered that when the converse experiment is done—slow growing clones generated in a wild-type background—the weaker cells are completely eliminated. Yet compartments (or indeed whole flies) consisting entirely of *Minute* cells form normally (Ferrus & Garcia-Bellido, *Wilhem-Roux Arch.* 183, 337; 1977). This phenomenon, termed cell competition by its discoverers, was discussed by P. Simpson (Gif-sur-Yvette) who suggested that the relative growth rate of the cells is the critical factor. *Minute* cells generated in a *Minute*

background, for example, survive. Further, starvation which reduces the total amount of growth, diminishes the effects of competition, rescuing the weaker *Minute* cells. It seems that some kind of cellular struggle which results in survival of the fittest is operating in growing cell populations.

J. Szabad (Szeged) reported results obtained in collaboration with T. Schüpbach and E. Wieschaus (University of Zurich). They have made gynandromorphs of *Drosophila* larvae and studied the distribution of male and female epidermal cells. These gynandromorphs are in many respects more valuable than adult ones, as they are a more direct representation of the blastoderm. Thanks to these studies we now know that the ♂/♀ boundary line on the blastoderm is convoluted, and that segmentation coincides with blastoderm stage. For a long time the relationship between the cells on the blastoderm and the gynandromorph fate map has remained uncertain. We now know that each cell on the blastoderm is represented by about 4 sturts on the fate map.

It is always debatable whether conferences are really worth it. In the case of Szeged there can be no question that the formal discussions, and more importantly, the informal ones (especially between colleagues who, for the wrong reasons, so rarely meet) were very worth while. □

The LEP summer study

from a Correspondent

MUCH progress in understanding subnuclear constituents has been made using a new facility for probing the structure of matter at extremely small distances ($\sim 10^{-14}$ cm), namely the electron-positron storage ring. In such a machine beams of electrons and positrons collide head-on producing a single massive photon, which can then pair produce other, heavier particles. This process is quite democratic; any charged particle which exists with a mass less than the equivalent energy of one of the electron beams must be produced with a calculable cross section. Furthermore the production process is 'clean'. The pair of new particles is made alone, devoid of the debris of other subnuclear particles which is always present if the proton beam of a conventional accelerator and a fixed target is used.

The basic constituents of matter seem to be fermions, occurring in families of four, each containing two quarks and two leptons. These fermions

Erratum

IN the article 'Illegitimate recombination legitimised' (*News and Views* 274, 213; 1978) the reference (column 3, page 213, line 10) to the 9-base pair repeat generated by the integration of insertion element IS4 and attributed to P. Starlinger, is incorrect. The repeated sequences generated by IS4 have not yet been determined.

interact through vector particles: the photon, charged and neutral massive vector bosons, and gluons which are responsible for the electromagnetic, weak and strong interactions, respectively. So far, experimental evidence for three families of fermions has been found: (u, d, e, ν_e), (c, s, μ , ν_μ), (t, b, τ , ν_τ). Of these, the charmed quark c, the bottom quark b, the heavy lepton τ and its neutrino ν_τ have only been discovered since 1974. The top quark t, though not yet observed, is confidently expected to be seen in the near future.

It was against the background of these exciting discoveries that some 80 particle and accelerator physicists from Europe and the United States met at the Summer School of Theoretical Physics in Les Houches, France and at CERN, Geneva from 11 to 22 September to study and discuss LEP, the proposed Large Electron Positron storage ring. The energy range of such a machine would extend from the highest energies attainable with the generation of machines now operational (PETRA at DESY, Hamburg, West Germany, see *Nature* 274, 202; 1978) or under construction (PEP at the Stanford Linear Accelerator Center in the United States), which might be ~ 30 GeV per beam, to energies of ~ 100 GeV and above. Much new and exciting information on heavy quarks and leptons is expected from PEP and PETRA. LEP however is mainly intended to probe the world of the vector particles, in particular the massive bosons W^+ , W^- , Z^0 which we believe are responsible for the weak interaction. Weinberg and Salam have proposed a possible unification of the weak and electromagnetic interactions in which both interactions have the same strength. The observed 'weakness' of the weak interaction results from the large masses of the W^+ , W^- and Z^0 as compared with the massless photon. Such theories predict the masses of the W^+ , W^- and Z^0 from measurements of weak interaction phenomena at low energies. So far the simplest such theory, the Weinberg-Salam theory, agrees with all observed weak phenomena, and predicts masses of ~ 90 GeV for the Z^0 and ~ 80 GeV for the W^+ and W^- . Because of the direct weak coupling of Z^0 to e^+e^- (recently experimentally observed in a beautiful experiment performed at SLAC: SLAC-PUB-2148, to be published in *Physics Letters*) an increase of 10^3 – 10^4 in the rate of interactions is expected if the beams of LEP are tuned to an energy of $1/2 M_{Z^0} = 45$ GeV. W^+W^- pairs of the above mass are also expected to be produced at a reasonable rate for beam energies in LEP of ~ 100 GeV. The observation of such

effects in LEP would mean that nearly a century after the discovery of weak interaction processes, their understanding would be on the same level as quantum electrodynamics (the only exact physical theory of particle interactions we have) and we shall have reduced from four to three the fundamental forces in nature.

In the intervening two years European particle physicists, represented by ECFA (The European Committee for Future Accelerators) under the chairmanship first of G. von Dardel, of the University of Lund, and more recently by M. Vivargent of LAPP, Annecy, have expressed a strong preference for such a large e^+e^- storage ring as the next major European high energy physics facility. During the same period a detailed design study of such a machine has been made (CERN/ISR-LEP/78-17). This design was the basis of the discussions at Les Houches. The storage ring is larger than any existing or planned accelerator, with a radius of 3.5 km. The estimated construction cost of ~ 1000 million Swiss francs is however somewhat less than that of the recently completed CERN SPS proton accelerator. In a preliminary time scale outlined by J. Adams, Executive Director General of CERN, the machine might become operational by about 1988, some 12 years after the start-up of the SPS. The construction of the machine within such a time scale seems possible without significant increase of the current CERN budget of some 600 million Swiss francs per annum provided some existing CERN facilities, by then becoming obsolete, were closed.

Much time was spent at the meeting on alternative 'scenarios' to the simple one described above. What if there were so many neutrinos coupling to the Z^0 that the width is much wider than the 3 GeV now expected? (a suggestion by J. Ellis, CERN). The answer seemed to be that floods of new charged heavy leptons must be seen at LEP. Some theorists (for example M. Veltman of the University of Utrecht) are already looking to even higher energies of more than 300 GeV, where effects of direct $Z^0W^+W^-$ coupling should become discernable. At this energy the currently quite mysterious mass spectrum of the quarks and heavy leptons should become considerably clearer. This is the world of the Higgs scalar boson which in the Weinberg-Salam theory is required to be an observable particle, but of unspecified mass. There were hints that the effects of gluons, the intermediaries of the strong interaction, should be seen particularly clearly in virtual $\gamma\gamma$ collisions, a process with a copious rate at LEP energies. Thus quarks and gluons could

one day possibly be liberated in the laboratory. In his concluding speech S. L. Glashow of Harvard University presented a dazzling catalogue of these alternative scenarios; a cornucopia of subnuclear riches. There have never been stronger arguments for building an accelerator before, and none has been as eagerly awaited as LEP. In the words of the motto chosen by the participants on their last evening at Les Houches, LEP's do it! $\square$

Breeding more protein

from B. J. Mifflin

Now you see it, now you don't. The protein gap much discussed in the late 1960s was largely abolished, at least on paper, by the lower protein recommendations of the 1973 FAO/WHO committee. Subsequently increased controversy has raged between those who argue that there is still a protein gap and those who protest that only calories are lacking. In many respects the arguments are futile and do little for those who have insufficient food which is of poor quality. The FAO/IAEA took a more positive position when in 1968 they organised a meeting to set up a co-operative programme with the GSF to improve the nutritional value of the grains of cereals and food legumes by supporting research programmes in a number of developing countries aimed at modifying protein and amino acid content. This September they convened a conference* to assess the results of the 8 years of the programme and review the present state of knowledge.

The continuing deficiency of adequate nutrition was emphasised in the opening talk by R. Bressani (Instituto de Nutricion de Centro America y Panama, Guatemala City). His results show that by up-grading the protein quality of cereal seed diets, either by using high lysine maize, or by adding legume seed supplements, food intake is increased, infant mortality reduced and the acreage required to provide sufficient protein considerably diminished. Since World Bank estimates indicate that more than a third of the world's population suffers from serious malnutrition the original aims of the programme still seem relevant and

* Organised by the Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture of the International Atomic Energy Agency (IAEA) and the Gesellschaft für Strahlen- und Umweltforschung (GSF) at Neuherberg, near Munich in the Federal Republic of Germany, on 4–8 September, 1978.

worthwhile; the remainder of the conference was chiefly concerned with reporting progress towards achieving those aims.

In the basic sciences considerable advances have been made. The major protein components of seeds are the storage proteins present in protein bodies. These are deficient in lysine in cereals and in sulphur amino acids in legumes. Although there are considerable differences between the storage proteins of legumes and cereals, in both cases they seem to consist of polymorphic families of related polypeptides. In maize, wheat and barley the putative structural genes have been located on various chromosomes and some mapping carried out. Success with *in vitro* synthesis of legumes and cereal storage proteins has been achieved in a number of laboratories and some mRNA has been isolated and cDNA prepared and cloned. These studies should provide a better understanding of the basic mechanisms of protein accumulation in seeds and offer the potential for use of genetic engineering techniques; some of these techniques were discussed in the context of future programmes.

Both as a direct result of the FAO/IAEA programme as well as of studies funded by other agencies, high lysine genes have been found in maize, barley, sorghum and possibly wheat and millet. The current status of the genes in the first three crops was reviewed. Although some progress has been made in understanding the nature of these mutations by determining their chromosome location little is yet known about their biochemical basis other than that the synthesis of the storage proteins is depressed. More information is required as to which of these genes offers the possibility of providing both high quality and high yield. So far the most promising breeding work seems to have been done in sorghum in which yield is not greatly affected, since the smaller size of the high lysine grains is balanced by increased seed number. Very promising results were also reported from CIMMYT, where the use of various modifying backgrounds have led to greatly improved *opaque2* lines of maize. Although some success has been made with the *Hipoly* gene in barley the lysine content of the lines seems to decrease as yields improve. Besides having normal yields new lysine varieties also have to be accepted by the consumers and meet the normal agronomic requirements, such as disease resistance. Although these criteria have not all been met it must be realised that plant breeding takes a long time and it is necessary to be patient. Some idea of the patience required was demonstrated by the

results of the Nebraska programme of high protein wheat breeding which had been in progress for 20 years before the release of the first variety in the US. This variety, which is now finding acceptance on the basis of its general properties, combines increased protein content and high yield.

In legumes the problems are more concerned with yield and protein quality than with protein content. In a critical and forceful talk H. K. Jain (Indian Agricultural Research Institute, New Delhi) pointed out the large amount of selection pressure and breeding that had been exerted on the cereals in comparison with the legumes. Legumes are not poor in total biological yield but insufficient of it is recovered in the harvested seed. Considerable success has been achieved through mutation breeding and recurrent selection programmes in obtaining improved ideotypes of several legumes with a much better harvest index. As regards protein quality, the problem is to improve the amino acid spectrum of the seed protein as well as to decrease the content of the various adverse nutritional factors present in legume seeds.

The conference ended with a review of the technological aspects of plant use and the role plant breeding and

biochemistry has played and must continue to play. Implicit in this must be an increased realisation that weight of produce alone is not enough and that a direct measure of productivity involves an assessment of the quality of the produce and the uses to which it can be put. When the work reported is viewed as a whole it represents a considerable success particularly in the time frame normally required to bring about stable genetic change and to select improved varieties of crop plants. It is therefore particularly disappointing that this programme is not being renewed with increased funding but, at best, will only continue in a limited way with funds from the Governments of the Federal Republics of Germany and of Sweden. Despite the fact that man's major necessity is food, all of which is ultimately derived from plants, the Plant Sciences remain amongst the Cinderellas, poorly funded and unfashionable. The FAO/IAEA programme has shown what can be done with a fraction of the funds spent on such grandiose technological playthings as supersonic travel. □

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A hundred years ago

M. BOUILLAUD, the once celebrated medical practitioner, who is a member of the Paris Academy of Science, assailed M. du Moncel in the sitting of September 30, and asserted that the phonograph and microphone experiments must be the work of ventriloquists. This fit of incredulity was occasioned by the recital of experiments made with the singing conductors. M. du Moncel asked for a commission of investigation to be appointed, although such accusations are not deserving of any notice, and have, indeed, raised universal ridicule. But the regulations of the Academy forbid any commission to be appointed to pronounce on the works or communications of members. Another curious scene took place at the sitting of last Monday. M. du Moncel presented to his colleagues, the "condensateur chantant," which had been

exhibited on the previous Saturday. He retired to the room of the Académie Française, in company with M. Faye, closed the door and sang. His voice was heard coming from a number of sheets of paper, in which six sheets of tinfoil had been inserted, and connected with the wires of an induction coil. M. Bouillaud was obliged to retreat from the position he had taken at the sitting of September 30. He made no allusion to the accusation of ventriloquism, but read a long quotation from Descartes, to show that "even if a speaking machine had been constructed, it could by no means be considered as a thinking machine." He said that speaking was not only a mechanical action, but also an intellectual work, so that neither the phonograph nor the singing condenser could be regarded by any means as really speaking! The whole assembly, in spite of its usual gravity, burst into roars of laughter. M. Milne-Edwards, who spoke at the previous sitting, said with much propriety, he should not have answered M. Bouillaud if he had understood such was his issue. Unfortunately he had understood, as everybody in the assembly did, that M. Bouillaud questioned the honesty of the experimenter.

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review article

Exploration of the continental basement by seismic reflection profiling

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The structure of the continental crust and uppermost mantle is being explored with higher resolution than previously attained through application of the seismic reflection profiling technique of the petroleum industry. Crustal structure is diverse, and features such as faults, unconformities, magma bodies, and the crust-mantle boundary have been delineated.

SOME four billion people derive their livelihood from this planet, so society's need for a thorough understanding of the Earth and a comprehensive inventory of potentially accessible Earth resources seems obvious. Yet except for mining prospects and districts, and the sedimentary basins that are the habitat of petroleum, there is little detailed or direct knowledge of subsurface Earth structure and composition at depths greater than a few tens or hundreds of metres. In most areas, deep rocks of the crystalline basement can be described only in general terms based on indirect evidence. Their genesis and evolution remain largely unknown.

The reasons for this remarkable state of affairs are partly economic and partly historical and organisational. They are also partly technological although there are means for improving substantially, if not ideally, our knowledge of the crystalline basement. Better mapping and improved field and laboratory studies of surface rocks, drilling for informational purposes, study of xenoliths of basement origin, and further application of a variety of geophysical methods are all feasible and promising. The article focuses on potentially the most productive and the most sophisticated geophysical technique—seismic reflection profiling—and its application to studies of the basement. This does not mean that other methods are not valuable; in general, they provide complementary information. The seismic reflection profiling technique discussed here should not be confused with the seismic refraction technique that is conventionally used for exploration of the crust, or with the Russian DSS method, which uses both refraction and wide-angle reflections.

During 1976 world expenditure on seismic reflection profiling was well over one billion dollars. Essentially all of this money was spent on exploration for petroleum. Continuing effort on this scale by the petroleum industry has resulted in, and is the result of, development of the technique of echo ranging in the Earth to a very high level of sophistication. In recent years, the industry has produced such innovations as non-explosive sources, digital field recording for movable listening arrays of thousands of detectors, computer processing of immense quantities of digital data, and powerful new methods of analysis and interpretation that have greatly enhanced understanding of the stratigraphy and structure of sedimentary basins. Modern reflection profiling bears little resemblance to the simple one shot—one detector method initially employed in the 1920s.

Within the past few years in several different countries, successful attempts have been made to apply these modern

techniques, with appropriate modifications, to exploration of the continental basement and hence greater depths (for a review see ref. 1). Here, the term 'basement' refers to crystalline rocks typically lying below the sedimentary veneer that is some 0–10 km thick. The continental basement rocks of interest here extend through the base of the crust at some 35–45 km and into the underlying mantle to depths of 50 or so km, the current limit of the method. Although rocks of the deep continental basement have been explored with some success previously using various geophysical techniques, the new efforts based on reflection profiling offer far greater resolution of structural detail and much better information on other properties that are currently unknown or poorly known. Because of the potential of this much improved, yet rarely applied, observational capability in seismology, the potential of other geophysical and geological methods, the great extent of the basement, and the direct and informative relation of the deep continental basement to the near-surface rocks, the continental basement must be considered one of the major scientific frontiers of modern geology.

The method

The principle of the seismic reflection profiling technique is deceptively simple. A disturbance at or near the surface of the Earth generates elastic waves that propagate through the Earth and are detected and recorded by sensors at the surface near the source. Although other kinds of waves are sometimes useful, most information comes from the compressional waves that travel along near-vertical paths and are partially reflected at buried sub-horizontal interfaces between rocks of contrasting impedance. The travel time of a pulse propagating along such a path is perhaps the prime information gathered, but the amplitude, phase, frequency content, and apparent surface velocity of the signal are also valuable.

The source and receivers are moved together along a line and the experiment repeated at closely spaced intervals so that subsurface reflecting horizons may be traced, and a profile or section obtained in a similar manner to that of a marine echo sounder. The basic travel-time data must be ultimately converted to depths and reflectors properly positioned; hence it is necessary to know or to measure the spatial distribution of velocities along the profile. One common method for velocity measurements uses reflected waves travelling along paths that are somewhat off the vertical. Thus, a single source might be detected and recorded by many sensors distributed over

distances ranging from near zero to significant fractions of the depth of the deepest reflecting horizon.

Furthermore, because the sources generate kinds of waves (surface waves, shear waves, waves with complex paths, diffracted waves) in addition to the nearly vertical travelling compressional waves that are normally the signal, arrays of many source elements and arrays of many receivers are commonly used to discriminate against the unwanted waves as well as background noise. Some data are also collected off the line, to permit the waves propagating out of the plane of the section to be identified.

In practice, there are numerous variations of the technique which depend on a large number of factors, such as local terrain, local propagation phenomena, economics, available hardware, characteristics of the particular target, and noise conditions.

As an example, a field procedure sometimes used in the COCORP project is described in some detail in the following. (COCORP is consortium for continental reflection profiling, and the project is the major US effort to apply the seismic reflection profiling technique of the petroleum industry to study of the deep basement of the continents. The procedure represents a compromise among various factors including acquisition of maximum possible information, economics, and logistics.)

COCORP uses the VIBROSEIS technique developed by the Continental Oil Company. Instead of an impulse generator such as an explosion, weight drop, or compressed air gun, a truck-mounted vibrator serves as a source element. Each vibrator imparts a peak vertical force of some 13 tons over about 20 ft² of the Earth's surface; the force is near-sinusoidal in time but of slowly varying frequency. The signal thus resembles a radar 'chirp' in character, though not in frequency. Typically the duration of vibration is 20 s, and the frequency varies linearly from 8 to 32 Hz over the interval. Cross correlation of the recorded signal with the input produces a record resembling that from an impulsive source, subject, of course, to restrictions of bandwidth and other properties of the VIBROSEIS source and the propagation. The advantages of the method include flexible and precise control of source character, elimination of drilling to implant explosives, avoidance of legal restrictions on explosives and the explosive hazard, and efficient coupling of source energy into elastic waves without great loss through rupture or other non-linear effects near the source. VIBROSEIS has been used widely and successfully on land by the petroleum industry for many years.

In a typical COCORP survey, stations in the field are spaced at 100-m intervals. Five vibrators operate synchronously 16 times at equally spaced intervals between stations, and the resulting signals are appropriately summed. Thus, 80 sweeps are injected into the earth per station interval and combined to form a source array 100 m long.

The receiving array includes 96 stations and is thus almost 10 km long. At each station a small spread of about 30 detectors, spaced to discriminate against natural and source-generated noise, is summed and recorded as one of 96 channels. Once the 80 sweeps at one station are recorded, the sources and the 3,000-element listening array are, in effect, moved 100 m along the line and the entire process repeated. Thus, along a line a few tens of kilometres in length, information travelling along tens of millions of rays is used in the analysis, a high level of redundancy that can be very advantageous. The data are recorded digitally in a form that will accommodate a very large dynamic range.

A further advantage is gained by computer processing the data. A wide variety and large number of methods including frequency and wavelength filtering, construction of synthetic seismograms, proper spatial positioning (migration), beam-forming, and various signal enhancement techniques are commonly applied depending on the particular problem. One powerful method, called common depth (or common reflection) point stacking, is based on combination of data from rays incident upon a particular reflection point at slightly different angles. Under favourable conditions signals are much enhanced.

COCORP typically uses 12, 24 or 48-fold common depth point stacking. In the sense that information from segments of many rays passing through a common zone is combined, the method resembles X-ray tomography.

Although seismic reflection data appear in other useful forms such as velocity profiles and contour maps, the end product is typically a section with distance as the abscissa and travel time, or depth, as the ordinate. For the casual observation, and to a first approximation, this may be thought of as a section through the Earth. It is misleading to carry this view too far, however. A detailed interpretation of the data requires an understanding of wave propagation, field procedures and data processing, and a knowledge of related geological and geophysical data for the area. This report is too brief to present detailed analysis or interpretation of data. Instead two sections are shown and described in general terms, and some conclusions based on studies to date are presented after a discussion of current efforts to explore the continental basement. (For a more detailed description of seismic reflection profiling techniques and interpretation as found in the petroleum industry see refs 2-5.)

Seismic exploration of the continental basement

A proper strategy for exploring a major frontier of the Earth sciences must include a judicious blend of several factors including the nature, scope and scale of the scientific problems, capability of the method, economics, expertise of personnel, and logistics. In an effort to achieve this blend, the COCORP project operates as follows. Many scientists from industry (largely the petroleum industry), government agencies, and universities participate in committees to advise on technical, scientific, and administrative problems. Cornell University acts as the operator or prime contractor. The field work is conducted by large geophysical contracting companies. Data are processed through routine stages, and available at modest cost, so that further specialised processing and interpretation are facilitated.

The choice and nature of sites for such surveying are critical. Six sites have been surveyed to date in the US. The first site in Hardeman County, Texas, was selected for favourable seismic and field conditions in initial testing of the method, and with little regard for the geology. Later sites, two in the Rio Grande Rift near Socorro, New Mexico, one crossing the boundary fault of the Wind River Mountains in Wyoming, one across the Franciscan basement-continental basement boundary in the Great Valley of California, and one across the San Andreas fault in California, were selected because of geological problems of special interest. A recognised problem of regional or crustal scale, as opposed to local or surficial scale, is found at each of these sites. Typically, a few lines of reflection profiling totalling some tens of kilometres have been carried out at each site. In all cases the results have been highly encouraging. In the longer term, however, it is planned that longer lines traversing the entire continent will be explored, so that problems of still larger scale will also be investigated. Applying this method of exploration to studying the modern basement is so new, and the region to be studied so vast and varied, that one must anticipate a period of rapid learning and development in the early stages of the project.

Two examples of the data

Figures 1 and 2 show two sections obtained by the methods described above for the locations in the Rio Grande Rift area^{6,7} indicated by 1 and 2A in Fig. 3. The reproductions do not do justice to the original data but some of the major features can be seen. The abscissa for Figs 1 and 2 is the distance in kilometres times 10; the ordinate is two-way travel time. Multiply by three to convert time to approximate depth in kilometres.

In Fig. 1, the upper 1-1.5 s of data for the western two-thirds of the profile show nearly horizontally stratified sediments of the rift valley. The sediments are broken into horsts and grabens by normal faults. It is only this portion of the section that would be

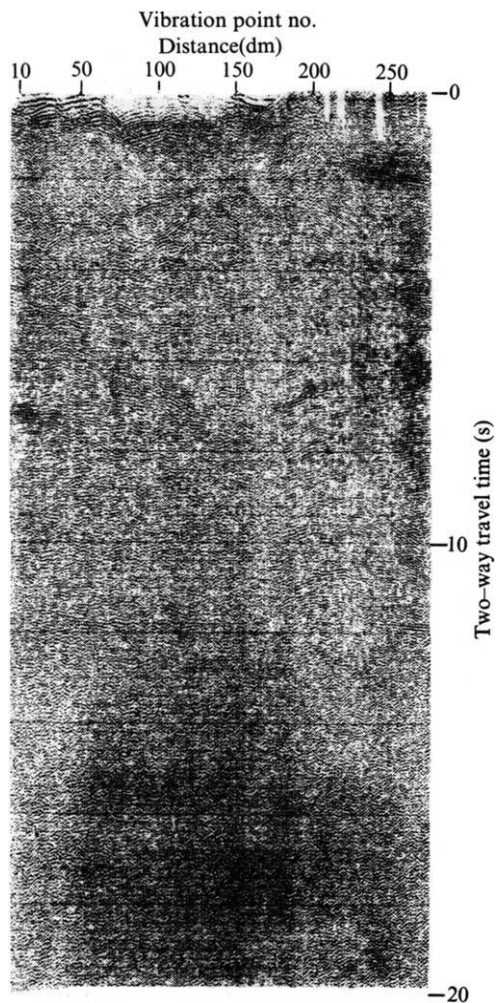


Fig. 1 Seismic section for line 1, Rio Grande Rift (see Fig. 3).

studied in normal petroleum exploration, and in much greater detail. Below these sediments is the crystalline basement which outcrops east of the bounding fault as seen at about station 180. Some shallow Palaeozoic sediments that overlap the basement at the eastern end of the section are indicated by events at <1 s.

Principal interest here is in information from greater depths. In some areas of the section, such as from ~ 1 to 3 s and between stations 10 and 75, there is a paucity of reflected data as indicated by the lack of short horizontal lineups. The rocks of such zones must be relatively free of acoustic heterogeneities. In this same range of times near the centre of the section are some horizons that dip to the west and may be faults. To the east (right) are additional basement structures.

Beneath the bounding fault through most of the record there is a zone lacking in reflected energy, possibly because of intrusives associated with the boundary. To the east (right) considerable structure in the basement is apparent, and at ~ 12 s there is a strong reflector that, based on time, corresponds to the top of the mantle. This reflector cannot be traced across the entire section, although it might be correlated with reflectors to the west at about the same time.

There is little reflected energy on this section at times greater than 12 s. At the western end of the profile there is a strong reflection at 7 s with an associated diffraction. This event corresponds to a horizon identified as the top of a magma body by Sanford⁸. Other profiles in the Rift show additional evidence for this mid-crustal body of molten rock. (For more detailed discussion of these data, see ref. 7).

Figure 2 shows a section for line 2A (Fig. 3) that begins slightly south of line 1 and continues southwards. Some of the major features are (1) the sediments showing much less structure than

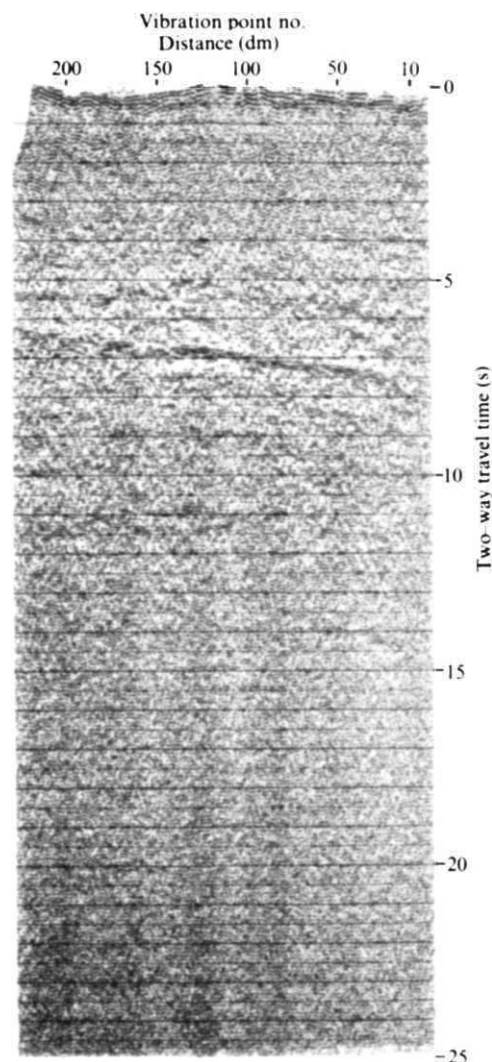
is the case for line 1 because line 2A is more nearly parallel to the geological structure, (2) a transparent zone in the upper basement between the base of the sediments and almost 4 s; (3) a strong reflector corresponding to the magma body at about 7 s and dipping to the north; and (4) a zone of reflected energy between about 11 and 12 s presumably corresponding to the top of the mantle.

The above qualitative and cursory descriptions obscure the great potential for obtaining more quantitative information by careful study, computer modelling, and other forms of analysis, but they provide a first impression of the type of data now available on the crust and illustrate the much improved resolution and vast potential of the technique.

Although seismic reflection profiling of the deep continental basement is relatively new, and only a tiny fraction of the basement has been surveyed at present, some conclusions can be drawn, although they must be held subject to revision as more data are acquired. The following points are based largely on COCORP experience, but they are compatible with similar observations elsewhere (see ref. 1 for a review and citations)^{6,7,9-13,16}.

(1) The continental basement at any particular site some tens of kilometres in dimension is typically heterogeneous on the scale of less than one or a few kilometres. Reflections from within the basement are only infrequently continuous for more than a few kilometres. Diffractions, reflection character, and transparent zones further attest to this heterogeneity. While layered models such as the classical but already outdated granite-basalt crust or

Fig. 2 Seismic section for line 2A, Rio Grande Rift (Fig. 3) for coordinates.



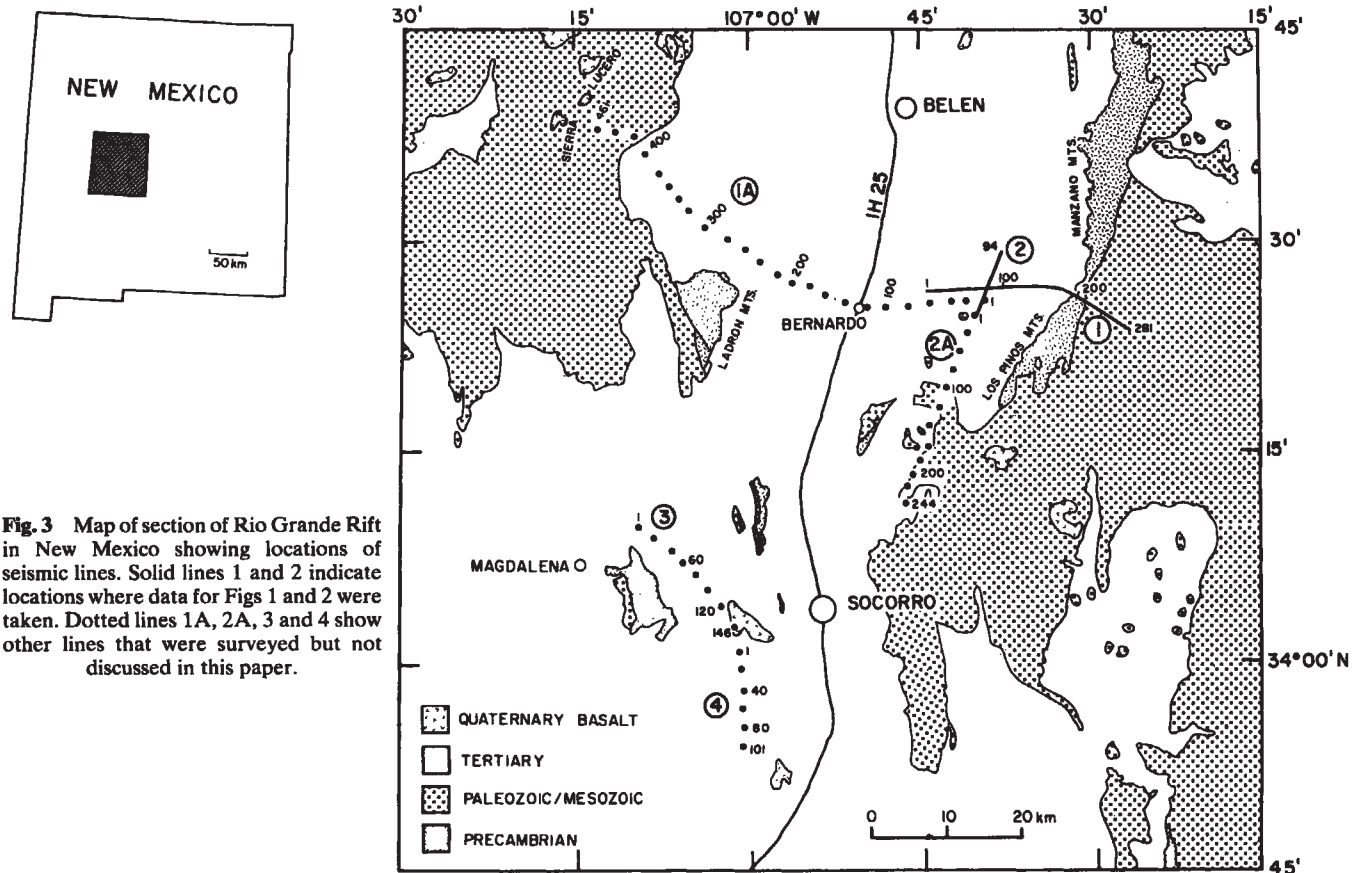


Fig. 3 Map of section of Rio Grande Rift in New Mexico showing locations of seismic lines. Solid lines 1 and 2 indicate locations where data for Figs 1 and 2 were taken. Dotted lines 1A, 2A, 3 and 4 show other lines that were surveyed but not discussed in this paper.

those based on seismic refraction data may be useful for interpretation of some seismic data, they can only be approximations in a gross sense to the geology of the crust. The deep crust seems to be, not surprisingly, like rocks with a history at depth but which are now at the surface (see also ref. 14).

(2) On a larger scale, there is great variation from site to site in the character of the seismic reflection profiles; consequently such variation must exist in the basement geology. With the exception of some features of the seismic profiles in the Rio Grande Rift, many of which show the magma body at a depth of about 18–20 km and some consistency at Moho depths, there is little similarity and great difference in seismic sections from one site to another. In Hardeman County strong diffractors are found in a quantity not observed elsewhere, and there are also shallow basement reflectors of unusual length of continuity, at least 17 km. The San Andreas fault in California shows up as a series of diffractors to depths of ~10 km, while deep reflectors are discontinuous across the fault zone. In the Wind River Mountain area the major thrust can apparently be traced to depths of almost 25 km. Many other deep reflectors corresponding to faults or other horizons may be seen. The Great Valley line shows fewest deep reflectors, perhaps because the Franciscan basement is so badly deformed. At any rate, there is little similarity from one site to another and ample evidence for de-emphasising universal models of the continental crust. It follows therefore, that a wealth of new knowledge of the structure of the crust must be available using the reflection technique.

(3) There is no evidence for a single consistent mid-crustal reflecting horizon corresponding to the Conrad discontinuity, a term that should probably be abandoned.

(4) In existing data the Mohorovicic discontinuity does not generally appear as a single simple reflector. Instead there is commonly a band of reflected energy. A possible interpretation is the laminated sort of boundary proposed by Meissner¹⁵ and perhaps suggested by exposures of the lower crust–upper mantle in the Ivrea zone.

(5) With the abundant evidence from geology and geophysics indicating heterogeneity throughout the crust, it would be remarkable if the mantle directly below the crust were homogeneous.

(6) At particular sites the method reveals features of the basement that, in nature, are geological (as opposed to geophysical) such as active magma bodies, faults, unconformities, folds, intrusions, and perhaps brecciated zones, as well as boundaries between rocks of different acoustical impedances. Detailed discussions of such sites can be found elsewhere^{9–13,16}.

(7) Continued application of the seismic reflection profiling technique including modern developments should provide a wealth of new knowledge and lead to a much enhanced understanding of the continental basement, a major frontier of modern earth science.

The results from the COCORP project depend on numerous participants from universities, government laboratories, and industry. This research was supported by the NSF.

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articles

Effects of intense stratospheric ionisation events

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High levels of ionising radiation in the Earth's stratosphere will lead to increased concentrations of nitrogen oxides and decreased concentrations of ozone. Changes in the surface environment will include an increased level of biologically harmful UV radiation, caused by the ozone depletion, and a decreased level of visible solar radiation, due to the presence of major enhancements in the stratospheric concentration of nitrogen dioxide. These changes are studied quantitatively, using the passage of the Solar System through a supernova remnant shell as an example. Some of the potential environmental changes are a substantial global cooling, abnormally dry conditions, a reduction in global photosynthesis and a large increase in the flux of atmospheric fixed nitrogen to the surface of the Earth. Such events might have been the cause of mass extinctions in the distant past.

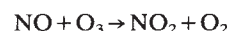
It has been proposed that¹ the observed correlation between extinctions of certain species of marine microorganisms (radiolaria and foraminifera) and geomagnetic polarity reversals could be explained by the occurrence of intense solar flares. Energetic solar protons ionise the Earth's stratosphere, producing nitrogen oxides as a by-product, and thereby depleting the ozone layer that shields the surface of the Earth from lethal solar UV radiation². At present the geomagnetic field confines the direct effects of solar protons to latitudes higher than about 60°, but during the brief periods of weakening or disappearance of the field accompanying a polarity reversal the global effect would be considerably more severe.

The history of life on Earth has been punctuated by widespread and apparently sudden (on the geological time scale) extinctions affecting both terrestrial and marine animals³. Examples of such massive extinctions, each involving a significant proportion of all species existing at the time, occurred at several of the major geological boundaries. The best known is probably the terminal Cretaceous extinction, which occurred about 65 Myr ago, but the fossil record has revealed several other extinction events of comparable severity, and suggests that

they have occurred at approximate intervals of 10⁸ yr. Various mechanisms have been proposed to account for these extinctions, including climatic changes, increasing ecological competition from newly evolving species, changes in sea level, disease epidemics, poisoning by volcanic emissions, increased radiation levels from nearby supernova explosions, and several others. None has proved entirely satisfactory, however, and it seems natural to ask whether the solar-flare mechanism proposed to account for the minor extinctions associated with geomagnetic polarity reversals could also account for these much more catastrophic and massive extinctions that have tended to mark many of the major boundaries of the geological record.

Solar flares

Reid *et al.*¹ discussed principally the increased UV dose received at the surface of the Earth as a consequence of the depletion of stratospheric ozone associated with hypothetical intense solar flares. Their calculations indicated that a solar flare 100 times more intense than that of August 1972 (the most energetic observed over the 20-yr period for which observations exist) would cause a global ozone depletion of about 50% and would increase the biologically effective UV dose received at the Equator by a factor of about 2.5, assuming that the flare occurred during the 10³–10⁴ yr duration of a geomagnetic polarity reversal. Although such an event would have serious enough consequences for living organisms, there is a second environmental effect that was mentioned only briefly. This is the reduction in solar radiation reaching the surface of the Earth in the visible part of the spectrum due to absorption by stratospheric NO₂, whose absorption cross section reaches a peak value of 6 × 10⁻¹⁹ cm² near 400 nm wavelength⁴. NO₂ is formed principally by the ozone-destroying reaction



and absorption of solar visible radiation provides a routine method of measuring the NO₂ content of the atmosphere^{5,6}. The calculations mentioned above indicated that the vertical NO₂ column content would become as large as 6 × 10¹⁶ cm⁻² during

daytime conditions several months after a solar flare 100 times more intense than that of August 1972. The consequent decrease in solar radiation reaching the surface at 400 nm wavelength for an overhead Sun would be about 3.5%, increasing to 7% for a solar zenith angle of 60°. The effect on the integrated solar radiation at the surface would be offset to some extent by the accompanying increase in UV radiation resulting from the destruction of ozone, but significant changes in the Earth's radiation budget, with accompanying changes in lower-atmosphere circulation, could be expected.

As with all suggested mechanisms, however, there remains a large element of speculation that is difficult to remove. Our knowledge of the flare mechanism itself is not sufficient to say whether energetic-particle fluxes 100 times more intense than those of August 1972 can ever be expected, even once in 10^8 yr, or whether there is some absolute limit beyond which a flare cannot go. Given the occasional occurrence of such super-flares, the hypothetical climate change runs up against the problem that the residence time of flare-associated NO_x in the stratosphere is only a few years, probably not long enough to allow the enormous heat reservoir in the oceans to adjust to the new conditions and produce a true climatic change.

Supernova explosions

The degree of speculation is reduced if we consider nearby supernova explosions as a cause of intense stratospheric ionisation instead of solar flares. The suggestion that mass extinctions could have been caused by supernova outbursts is an old one, and was revived by Terry and Tucker⁷, who discussed the potential radiation hazards of such an event. Since then, other consequences of nearby supernova explosions have been suggested. Russell and Tucker⁸ specifically raised the possibility of a climatic change associated with such an event in connection with the terminal Cretaceous extinctions, though without discussing any mechanism. Ruderman⁹ and Whitten *et al.*¹⁰ discussed the depletion of stratospheric ozone expected from nearby supernova explosions, and Hunt¹¹ has recently estimated the surface temperature change caused by this depletion.

Clark *et al.*¹² have suggested that a supernova is likely to occur within 10 pc of the Solar System approximately once in 10^8 yr during passage through one of the spiral arms of the galaxy. At a distance of 10 pc, the Solar System would probably lie within the supernova remnant (SNR) shell for a period of several hundreds of years. There is growing evidence that SNRs are the source of galactic cosmic rays¹³ and that the cosmic rays are accelerated continuously during the lifetime of the SNR. This point of view is

consistent with indirect evidence from the radio emission of SNRs that they contain a cloud of cosmic-ray particles with a total energy of the order of 10^{50} erg, leading to a cosmic-ray flux of the order of 100–1,000 times the present background level.

Model of stratospheric photochemistry

We have used a one-dimensional time-dependent model of stratospheric photochemistry¹⁴ to study the effect of such enormously increased cosmic-ray fluxes on the total solar radiation penetrating the stratosphere. The model calculations were started with initial conditions corresponding to global average present-day conditions, and with all photochemical mechanisms appropriate to a latitude of 30°. The enhanced cosmic-ray fluxes began abruptly on 1 January, and were kept at a constant level for the duration of the calculation, which was taken as 5 yr.

Figure 1 shows the annual average values of total vertical ozone column content for cosmic-ray fluxes 100 and 1,000 times the present value, and indicates that a steady state has been reached after about 5 yr. Total ozone depletions amount to 64% and 89%, respectively, and are considerably larger than those calculated by Whitten *et al.*¹⁰ The differences apparently arise from differences in the model parameters, and in particular in the vertical eddy-diffusion profiles. All model calculations can only be approximate for such major perturbations, however, as it is impossible to estimate the effect of major compositional changes on transport coefficients. Figure 2 shows the corresponding values of NO_2 vertical column content, again indicating a near approach to a steady state after 5 yr, with increases from the present level of $4 \times 10^{15} \text{ cm}^{-2}$ by factors of 13 and 130, respectively.

Fig. 1 Vertical column content of ozone as a function of time after the onset of enhanced cosmic-ray fluxes.

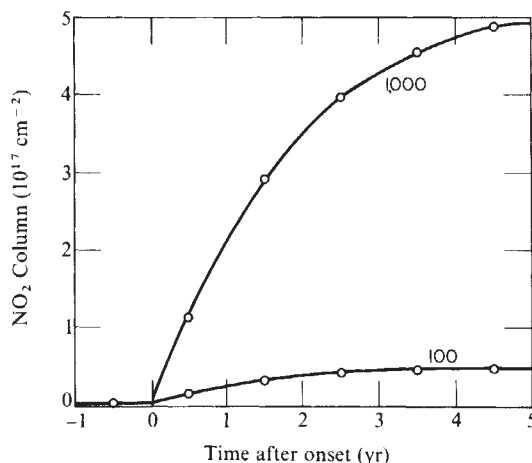
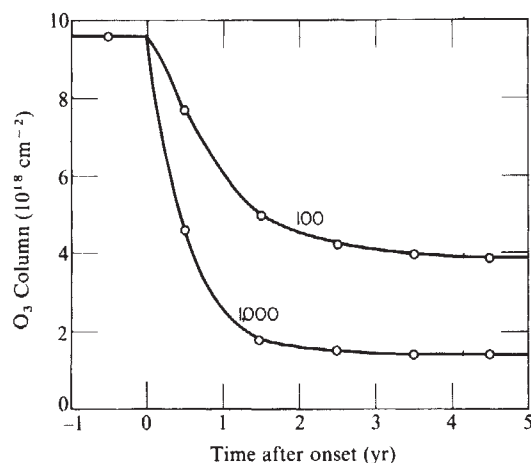


Fig. 2 Vertical column content of NO_2 as a function of time after the onset of enhanced cosmic-ray fluxes.

Figure 3 shows the annual average steady-state height profiles of O_3 and NO_2 concentration for present-day conditions and for the same two cases of enhanced cosmic-ray flux. Concentrations below 10 km have been omitted because the appropriate tropospheric and surface sources are unknown; their effect on the stratospheric concentrations is negligible. The major depletions of O_3 and enhancements of NO_2 occur in the lower stratosphere, where the photochemical lifetime of ozone is long, and where cosmic-ray ionisation peaks. For the case of a 1,000-fold increase in cosmic-ray flux, the NO_2 concentration approaches that of ozone near 20 km.

As mentioned above, the model calculations are carried out for 30° latitude conditions, and can be taken to represent a global average. In estimating the effects at latitudes other than 30°, we assume that the stratospheric composition is the same everywhere. This assumption would, of course, be invalid if we

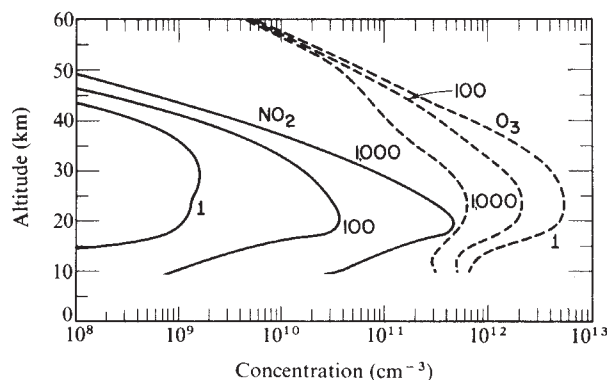
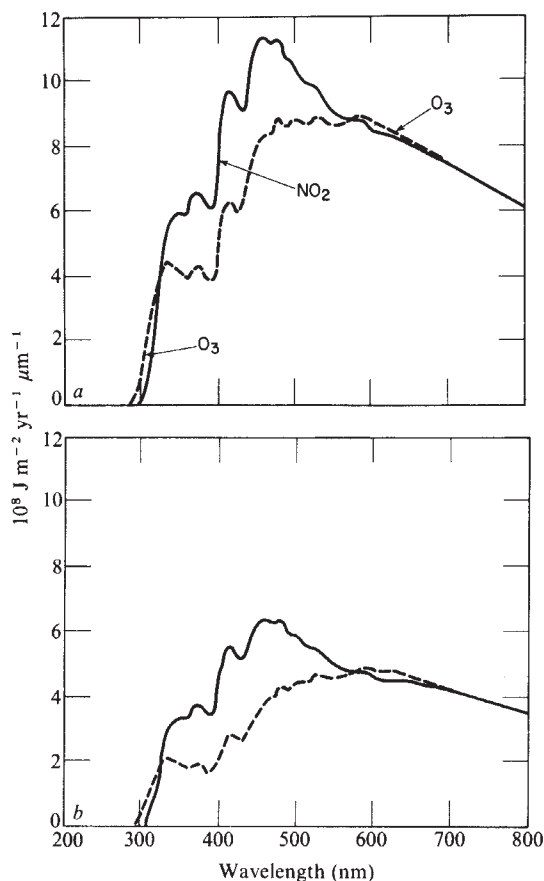


Fig. 3 Vertical altitude profiles of O_3 and NO_2 for present conditions (1) and for enhanced cosmic-ray fluxes (100 and 1,000).

were concerned with absolute values, but it allows us to estimate approximately departures from normal conditions. Figure 4a and b shows the spectral distribution of direct solar radiation penetrating the stratosphere throughout one year for latitudes of 0° and 60° , respectively. The solid curves represent present-day conditions, and the broken curves refer to steady-state (that is 5 yr after onset) conditions for the case of a 1,000-fold cosmic-ray enhancement. Modifications to the spectrum occur in three distinct spectral regions: the annual solar radiation is increased in the near UV, at wavelengths shorter than 325 nm,

Fig. 4 a, Direct solar radiation penetrating the stratosphere at the Equator integrated over one year (solid curve, present-day conditions; broken curve, steady-state conditions for a 1,000-fold cosmic-ray enhancement). b, Same as (a) for latitude 60° .



due to the decreased absorption in the Hartley and Huggins bands of ozone; blue and green visible radiation (325–570 nm) is substantially decreased, due to the increased absorption by NO_2 ; longer wavelength visible radiation is slightly increased, due to the decreased absorption in the Chappuis bands of ozone. The net environmental effects are (1) an increase in the biologically effective dose received by living organisms at the surface; and (2) an appreciable decrease in the total integrated solar radiation available for heating the lower atmosphere and the surface.

Figure 5 shows the fractional change in annual integrated direct solar radiation penetrating the stratosphere at the indicated latitudes for the two cosmic-ray flux enhancements. In the case of the 100-fold enhancement, the integrated radiation is increased by a few tenths of a per cent, as the increased absorption by NO_2 is not sufficient to compensate for the decreased ozone absorption. In the case of the 1,000-fold enhancement, however, the increase in NO_2 absorption overwhelms the decrease in ozone, and the integrated solar radiation is decreased by several per cent at all latitudes.

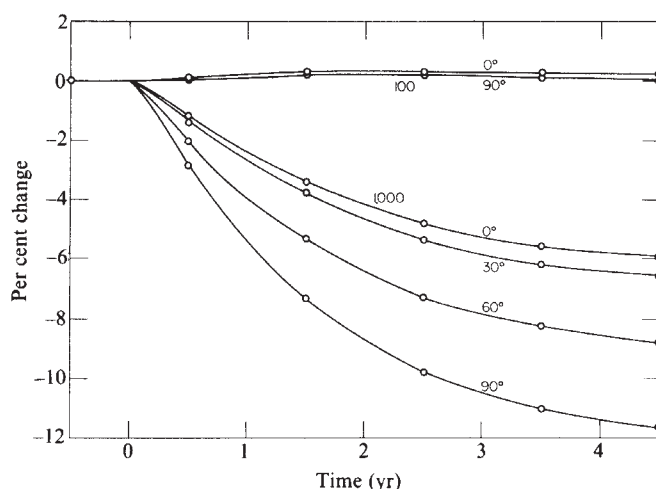


Fig. 5 Direct solar radiation penetrating the stratosphere integrated over one year and over all wavelengths.

In attempting to estimate the climatic effects of these changes in integrated solar radiation, it is important to realise that they are not equivalent to changes in the solar constant of the same magnitude. The radiation that is prevented from reaching the lower atmosphere in the 1,000-fold enhancement case is absorbed in the stratosphere, where it greatly increases the heating rate and thus the temperature, leading to a substantial increase in stratospheric thermal radiation. This partially compensates for the deficit in direct solar radiation at the surface. There will also be a change in the effective albedo of the lower atmosphere as a result of the change in spectral composition of the incident radiation.

These perturbations in the terrestrial radiation budget will certainly be accompanied by important changes in the dynamic coupling between the stratosphere and troposphere, and a calculation of the full climatic consequences is not yet possible. Estimates made with a relatively simple radiative-balance model, however, predict a drop in global average surface temperature of about 3 K for the 1,000-fold enhancement case. The complexities of radiative feedbacks are such that this figure is significant only as an indication of a major climatic perturbation, but is much larger than that estimated by Hunt¹¹, using a more sophisticated radiative-convective model. Hunt's calculation, however, included only the effects of the ozone depletion, and did not consider the much larger effect of the enhanced NO_2 that must accompany the depleted ozone.

Despite the qualitative nature of the estimated global cooling, some of the consequences are worth exploring. A 3 K drop in surface temperature, for example, would lead to a drop in global average atmospheric water-vapour content of about 20%, assuming a constant average tropospheric relative humidity profile as quoted by Manabe and Wetherald¹⁵. This would decrease the greenhouse effect, tending to decrease surface temperatures even more and would also lead to changes in cloud properties, with further climatic consequences. The reduction in water-vapour content might be expected to lead to widespread drought conditions, but might prevent the occurrence of a true 'ice age', as above-normal precipitation is probably required for the development of continental ice sheets.

Figure 6 shows the increase in the biologically effective UV radiation penetrating the stratosphere in the course of a year at the indicated latitudes for the two cases. These values were obtained by multiplying the integrated annual radiation at wavelengths between 280 and 310 nm by a 'biological effectiveness' factor¹⁶ derived on the basis of the absorption cross sections of protein and nucleic acids, and integrating over wavelength. Increases in UV dose by more than an order of magnitude are indicated, but these should be regarded with caution, as the effects of Rayleigh scattering by the lower atmosphere, which are substantial for UV wavelengths, have not been included. The results are thus only semi-qualitative, but indicate a severe increase in UV radiation levels.

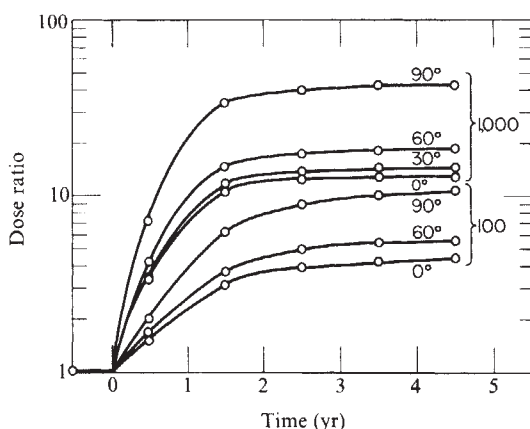


Fig. 6 Biologically effective annual UV dose during periods of enhanced cosmic-ray flux. Quantity shown is the ratio of the enhanced dose to that received in present conditions. Transmission through the lower atmosphere has not been included in either enhanced or normal dose.

The reduction in visible radiation intensity resulting from the 1,000-fold enhancement case is also likely to have an effect on global photosynthetic activity, as noted previously by Crutzen and Reid¹⁷. Photosynthesis is most efficient at wavelengths in the blue (near 450 nm) and in the red (near 660 nm), and Fig. 4a shows a reduction in the important blue light of about 30%. The integrated annual radiation in this wavelength region received at the Equator is thus equivalent to that received in present conditions at 40° latitude, and the radiation received at 40° is equivalent to that now received at 60°. Coupled with the probable drought conditions resulting from the decrease in global temperature, a severe decrease in the abundance of plant life is probable. According to our present understanding of the terrestrial carbon cycle, the decrease in plant life would probably lead to an increase in atmospheric CO₂ concentrations, which in turn would tend to increase the surface temperature through the greenhouse effect. The temperature might become stabilised in this way at a value somewhat above that predicted in the absence of the biosphere, but with a globally reduced biomass.

A further perturbation to the biosphere might arise from the increased precipitation of fixed nitrogen on the surface of the Earth. The ultimate fate of fixed nitrogen diffusing into the troposphere from above is probably rainout leading to surface deposition. The model calculations show that the total downward flux of N at tropopause heights is about 2×10^{10} kg yr⁻¹ for the 100-fold enhancement case and 2×10^{11} kg yr⁻¹ for the 1,000-fold enhancement case. The former value is comparable with current estimates for the annual amount of nitrogen fixed in the atmosphere by lightning¹⁸, and the latter value is comparable to the total amount of nitrogen fixed by biological processes¹⁹. The addition of such large quantities of biological nutrient would have an impact on the biosphere that is not easily estimated.

Conclusions

We may conclude that passage of the Earth through a SNR would be likely to cause a prolonged period of harsh environmental conditions for the biosphere, including high levels of damaging UV radiation, cooler global temperatures and generally dry conditions (depending on how intense the cosmic-ray flux within the SNR becomes), and reduced photosynthetic activity. Whether or not these severe conditions would lead to major extinctions of animal populations is a matter for speculation. The evidence from the geological and fossil record is not clear, although McLean²⁰ has interpreted the Cretaceous-Tertiary record as supporting a global warming, rather than a cooling, as the cause of the terminal Mesozoic extinctions. If this view is correct, a nearby supernova event can probably be eliminated as the cause of these particular extinctions.

The probability of a nearby (within about 15 pc) supernova explosion is also debatable. At the low end of the scale, Whitten *et al.*¹⁰ have estimated a 1–5% chance of a supernova within 5–10 pc in a period of 10⁸ yr. Data presented by Shklovsky²¹ lead to an estimated frequency of one in 1.5×10^8 yr at 15 pc distance, while Clark *et al.*¹² estimate one in 10⁸ yr at 10 pc distance on the basis of the confinement of type 2 supernovas to the vicinity of the spiral arms of the Galaxy, and the regularity of the passage of the Solar System through these spiral arms. Whichever view is correct, a nearby supernova explosion is not a dim and exotic possibility when viewed in the geological time scale, but rather an event that may well have occurred several times within the 10⁹-yr lifetime of the biosphere.

Finally, note that enhancements in cosmic-ray flux by a factor of 1,000 lasting for 1,000 yr would cause an enhancement in the average cosmic-ray flux over a 10⁸-yr period of only 1%. Such events would therefore be quite consistent with evidence from lunar rocks and meteorites that the average cosmic-ray flux has varied by less than a factor of two over the past 10⁹ yr (ref. 22).

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The origin of labyrinth and tower karst and the climatic conditions necessary for their development

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A new karst style, labyrinth karst, is recognised in climates ranging from humid tropical to subarctic. In the late stages of evolution it is replaced by limestone towers indicating that tower karst is polygenetic, and not specific to the humid tropics as was once thought.

THE morphoclimatic principle that specific climates produce specific landforms has been more frequently applied in the study of karst (solutional) landscapes than in other branches of geomorphology. The impetus for its adoption came from pioneer studies of humid tropical karstlands in the 1930's¹. These revealed a wider variety of forms developed on a grander scale than elsewhere, and two broad karst styles—cone karst (Ger. *Kegelkarst*) and tower karst (Ger. *Turmkarst*)—that did not seem to develop in temperate or colder areas. In later work climate has been called upon to explain both areal and altitudinal variations in the character of karstlands. Despite reaction to what some consider as a grave overemphasis of climatic factors^{2,3}, the principle is still widely accepted. Central to that acceptance is the supposed humid tropical climaspecificity of the cone and tower karst styles, for which thresholds of temperature and precipitation have been proposed (Table 1).

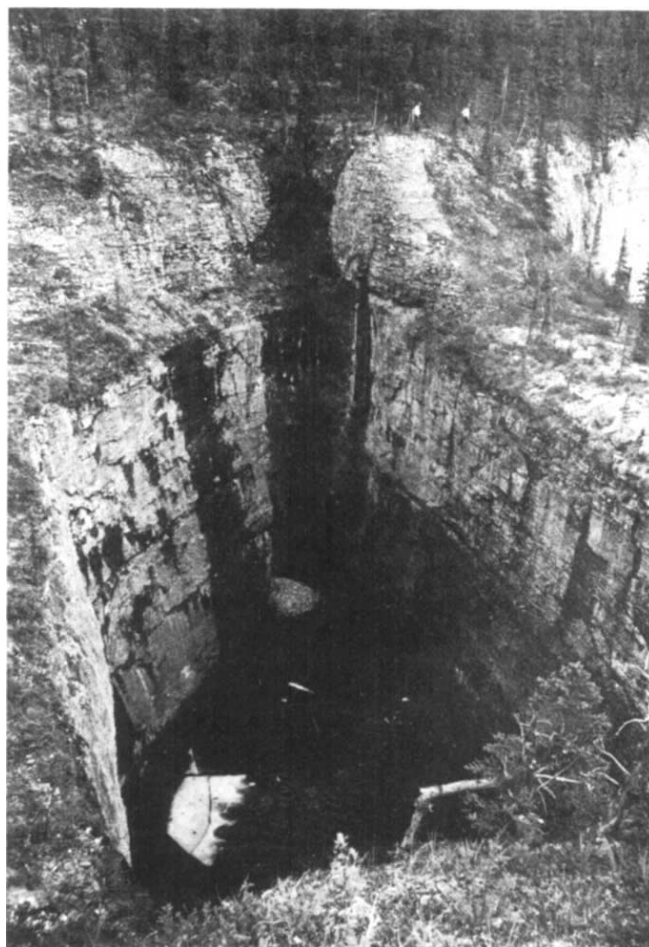
In 1972–74 we explored and studied a complex and accentuated karstland at lat 61–62° N in the Nahanni area of the Mackenzie Mountains, Northwest Territories, Canada. Landforms are at an altitude of 1,000–1,300 m. The climate is of the subarctic continental interior type and there is discontinuous permafrost. Our findings have added to knowledge of karst styles and suggest that some relationships between karst morphology and climate be reassessed.

The Nahanni karst is dominated by a series of vertical-walled landforms which are linked in a simple yet remarkable developmental sequence^{12,13}. During early stages water draining underground via vertical fissures creates strings of solution dolines, circular to elliptical in plan view and cylindrical in depth (Fig. 1). By enlargement and coalescence along the fractures, strings of dolines are converted to intersecting networks of karst streets. As streets deepen and widen, the intervening rock ridges are dissected and ultimately destroyed. Replacing them are large closed depressions of angular planform and irregular distribution that we term 'karst platea'. Where insoluble sediment is carried to these depressions by allogeneic streams, underground drainage becomes impeded and there is flooding after heavy summer rains. Floors are alluviated and the platea become small poljes. As platea and/or poljes expand, rock towers are left which rise from uneven karst margin plains. At this stage of evolution the landscape resembles a scaled-down version of the spectacular tower karsts of Puerto Rico or south

China. In the north of the Nahanni region karst streets, platea, and poljes form an intricate natural rock labyrinth (Fig. 2). Streets are as much as 185 m deep and 9 km long, and platea and poljes up to 125 m deep and 2–3 km long. Limestone towers with vertical to overhanging walls are up to 125 m high (Fig. 3).

We have identified karst landscapes like that of the Nahanni in several humid and seasonally humid tropical carbonate terrains. In south-west Celebes, Sunartadirdja and Lehmann¹⁴ describe

Fig. 1 The early doline stage of labyrinth karst development. Rappel Cenote is an elliptical-shaped doline 30 m deep. Even in summer drainage routes are blocked by subsurface ice so that ponds are common in such depressions.



karst streets 6–400 m wide, 50–200 m deep, and up to 8 km long. Where these intersect, depressions (platea) have developed. Several have been alluviated by influx of insoluble residue from nearby crystalline rock indicating that they are the embryos of future poljes. Scattered, isolated limestone towers are also common. Similar topography is found on many upland surfaces in Sarawak¹⁵. For example, at Subis joint-guided corridors (streets) are about 30 m apart and 3–150 m deep. At intersections depressions (platea) 5–180 m deep and 5–500 m across are common. At an advanced stage of dissection rows of tall 'needles' (towers) are all that remain between the streets. Stereo photographs (Plate 2 of ref. 15) reveal a rock labyrinth nearly identical to that in the Nahanni. Deep, intersecting karst streets several kilometres long are reported in the Star Mountains and Lake Ajamaru regions of New Guinea^{2,16}. They are also clearly visible in oblique photographs of the Sierra Maestra in Cuba (Fig. 16 of ref. 3), and of the 'Ruined City' of Arnhem Land, Northern Territory, Australia (Fig. XIV-24 of ref. 17) where they isolate numerous pinnacles, towers, and ridges. Less spectacular labyrinths are known in the Tanga region of Tanzania where streets and towers have a maximum relief of 15 m (ref. 18), and in Yunnan, south China, where corridors with ridges and pinnacles up to 30 m high form a landscape that has been described as a "tropical rock forest"¹⁹.

Natural rock labyrinths and associated tower karst are also described in tropical semiarid areas. In the Limestone Ranges of the Fitzroy Basin, Western Australia²⁰, intersecting networks of karst streets up to 6 m wide, 30 m deep, and hundreds of metres long form large areas of 'dissected karst' which include enclosed depressions of angular planform up to 1.5 km long, 180 m wide,

Fig. 2 Aerial photograph of the Nahanni showing labyrinth karst terrain with streets, platea, poljes, and isolated towers.

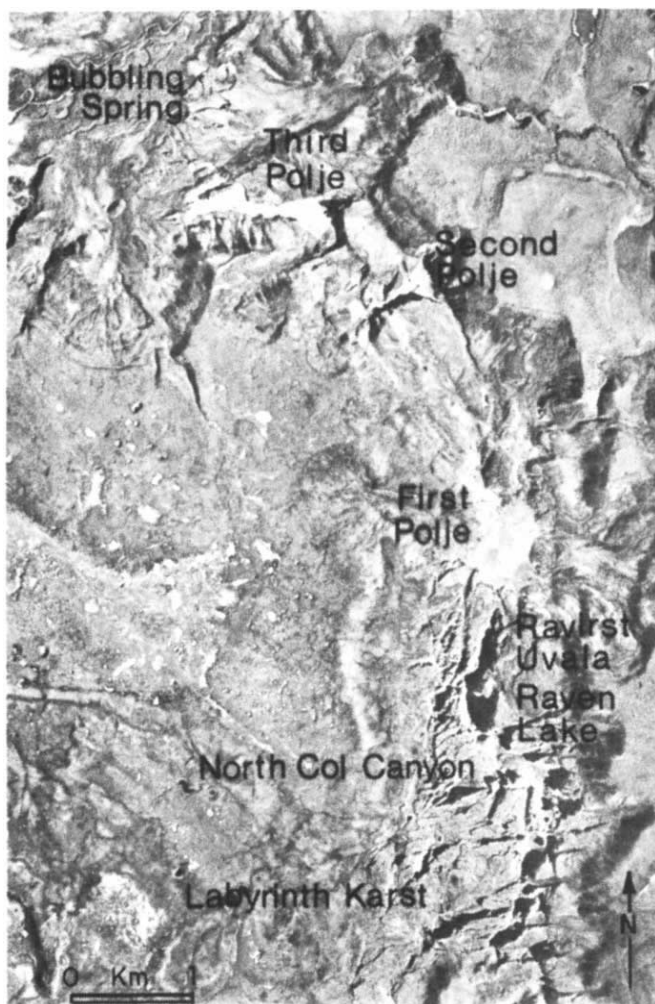


Fig. 3 A late tower karst stage in the evolution of labyrinth karst in the Nahanni. Insel Tower is approximately 65 m high and rises from a flat alluviated plain.

and 30–45 m deep. These have steep to vertical walls, and flat floors almost entirely mantled by alluvium or residual soil. Jennings and Sweeting²⁰ refer to them as "miniature poljes" but they may well be the semiarid equivalents of karst platea. In addition to the dissected karst are areas of towers, pinnacles, and narrow ridges up to 45 m high which rise from a rock pediplain. The karst of Bom Jesus da Lapa, Brazil, in a limestone hill 2 × 1 km and 200 m high, is similar to the Fitzroy terrain²¹. The hill is crossed by street networks 1–2 m wide and 10–30 m deep which isolate limestone pinnacles, ridges, and towers. Also common are circular solution pits 2–5 m in diameter and 10–30 m deep which appear identical to the dolines that form during the early stage of karstification at Nahanni.

The existence of such similar rock labyrinths in widely distributed carbonate terrains, and of shallower street networks elsewhere^{22–29}, demonstrates that a new karst style—'labyrinth karst'—must be recognised. This style can develop at a variety of scales, with individual depressions ranging from several centimetres to a few hundred metres in depth¹³. During the later stages of evolution a tower karst landscape emerges either directly from karst street networks, or through intermediate platea or platea and polje stages. Variations in morphology between labyrinths are slight and reflect differences in lithology, climate, and in the availability of insoluble residue. The climatic effect is minor. For example, in the Nahanni karst, depression floors and walls are frequently mantled by accumulations of frost-shattered limestone while in the hot semiarid Fitzroy Basin of Australia, where pedimentation processes influence karst evolution, rock debris is uncommon.

In many tropical karstlands two successive stages of erosion can be recognised^{4,5,10}. Cone karst develops until the floors of cockpits extend to local base levels and significant quantities of alluvium are retained. Thresholds between adjacent depressions are submerged, and cones are left standing above broadening alluvial plains. Lateral solutional undercutting may then transform the cones into steep-sided limestone towers. Because true tower karst also forms during labyrinth karst evolution, the tower karst style is polygenetic³. It forms from cone karst when solution is concentrated in a single horizontal plane and from labyrinth karst because solution is concentrated in discrete vertical planes. In the one case tower karst is a conical karst formed under special hydrological, structural and lithological conditions^{6,7,30}, in the other it is entirely a product of particular structural and lithological characteristics.

In the Lake Ajamaru region of New Guinea Pannekoek¹⁶ has identified all stages in a karst cycle of erosion that commences with the formation of small surface valleys aligned along major joints. As water begins to drain underground joints are widened and clefts (karst streets) are opened, isolating angular limestone hills. At this stage the landscape conforms to labyrinth karst. As

Table 1 Suggested temperature and precipitation thresholds for cone and tower karst development

Source	Temperature threshold (°C)	Precipitation threshold (mm)	Area of applicability
Sweeting ⁴		1,840*	Jamaica
Gerstenhauer ⁵	high	1,000	Worldwide
Verstappen ⁶	17–20*	1,500	Worldwide
Balázs ⁷	18	1,200	Worldwide
Sweeting ⁸	18*	1,480*	South China
Williams ⁹	13*	3,250*	New Guinea
Jakucs ¹⁰	17–18	1,000–1,200	Worldwide
Monroe ¹¹		1,300*	Puerto Rico

* Values estimated from statements made by the various authors or from information given by them.

the clefts continue to widen the hills become smaller and lose their angularity so that Kegelkarst topography is produced. Karst on the northern flank of the Sierra Maestra, Cuba (Fig. 16 of ref. 3) seems to fit well into Pannekoek's cycle; residual towers and ridges within a labyrinth are becoming rounded and a landscape resembling oriented cone karst (Ger. gerichteter karst) is emerging. The indication is therefore, that like tower karst, cone karst is polygenetic and that in some areas labyrinth karst may be an early stage in its development.

Once the basic characteristics of cone and tower karst became known in the 1930's and 1940's, examples were reported well outside of modern humid and seasonally humid tropical limits^{31–34}. In almost every case these landscapes were interpreted as fossil, survivals from former periods of tropical climate. For example, Tricart and Da Silva²¹ maintain that the Bom Jesus da Lapa karst was fashioned in a humid Tertiary climate and that it has suffered only minor modification in the much drier conditions that have existed since the late Miocene. A similar interpretation has been put forward for mogotes in the Cracow Upland of Poland³¹, which are identical to the towers of Nahanni, and which are scattered over a similar-sized area (15 × 5 km). A fossil or palaeoclimatic interpretation of the Nahanni karst is untenable. Evidence such as the presence of abundant erratics from the Canadian Shield indicates that the area was glaciated more than once during the Pleistocene. The present karst landforms are developed from a glacially scoured limestone surface. They post-date the last Laurentide ice to cover the area. The karst therefore seems to have originated in climatic conditions that, on average, were at least as harsh as those of today—that is a mean annual temperature of –4.5°C and a precipitation of only 566 mm. These are well below the minimum conditions supposed necessary for tower karst development (Table 1).

Recent palaeogeographical work in Australia also shows that labyrinth and tower karst can form in cold climatic conditions. In the partly glacial Lower Permian sequence of the Carnarvon Basin, Western Australia is a locally-exhumed karst landscape covering approximately 1,000 km². Karst streets up to 20 m wide, and pinnacle-shaped towers up to 30 m high are developed in calcarenite of the Callytharra Formation. A thick tillite-rich sequence underlies the Callytharra and the karst is overlain by marine sediments containing rare boulder-size erratics. In addition, all Lower Permian faunas from this region indicate cool to temperate conditions (van de Graaff, W. J. E. Hocking, R. M. and Denman, P. D., Geological Survey of Western Australia, personal communication).

Recognition of labyrinth and associated tower karst in climates ranging from humid tropical to dry subarctic suggests that, like doline karst, these styles are not primarily climate or latitude dependent (Table 2). Why then do deep labyrinths and isolated towers develop in some areas and not others, and why are they more common in the tropics? Two local factors seem to have been important in the formation of the Nahanni labyrinths. First, this area was beyond the limits of the Wisconsinan (last) Laurentide ice sheet, and may even have escaped glaciation during the Illinoian period³⁶. It is one of few high-latitude carbonate regions where karst has had time to develop fully, without drastic interruption by flowing ice. Second, although mean soil P_{CO_2} (0.3%), solution intensity ($CaCO_3$ 144 mg l⁻¹), and solute denudation (17.9–27.1 m³ km⁻² yr⁻¹), are much lower than in the tropics, (where the average soil P_{CO_2} at 30 cm depth is 2.0%³⁷, mean water hardness lies between 165 and 180 mg l⁻¹ $CaCO_3$ ^{37,38} and estimated denudation rates include 83 m³ km⁻² yr⁻¹ for Indonesia³⁹, 50–60 m³ km⁻² yr⁻¹ for the Philippines⁴⁰, and 72 m³ km⁻² yr⁻¹ for Jamaica⁴¹) solutational activity is not constant across the area. It seems significant that the deepest solutational landforms are located in a zone that receives large volumes of acid allogeneic water from nearby shales, and that elsewhere only shallow karst street networks are encountered. Annual denudation in this zone is two to three times the regional average bringing it close to rates typical of tropical cone and tower regions. At the present rate—approximately 50 m³ km⁻² yr⁻¹—it is estimated that the Nahanni landscape could have formed within the last 200,000 yr, a period that has been available for uninterrupted karst development.

The local factors explain why the Nahanni karst is more highly developed than other subarctic carbonate terrains but not why labyrinth and tower karst predominate, rather than other styles such as doline karst. Many temperate karstlands have longer uninterrupted histories of evolution than the Nahanni, and display greater solute concentrations, runoff and denudation rates than some humid tropical regions³⁷. Yet they lack towers and networks of deep karst streets. Total denudation, therefore, does not determine labyrinth karst development; it seems that structural and lithological properties of the host rock are

Table 2 Areas and climates in which deep labyrinths and late-stage tower karst have been discovered*

Area	Ref.	Approximate latitude	Köppen climate	Temperature (°C)	Precipitation (mm)
Maros, Celebes	14	5°S	Af	26.4	2,850–3,360
Lake Ajamaru, New Guinea	16	2–4°S	Af	26.6–28.3	2,490–2,845
Star Mountains, New Guinea	2	3–4°S	Af		
Arnhem Land, Australia	17	12–14°S	Aw	26.6	1,070
Sierra Maestra, Cuba	3	20°N	Aw	25.3	1,090
Tanga, Tanzania	18	5°S	Aw	26.0	1,350
Bom Jesus da Lapa, Brazil	21	9–11°S	BShw	26.5	700–900
Fitzroy Basin, Australia	20	17–19°S	BShw	27.6	460–710
Carnarvon Basin, Australia	†	50–60°S‡		Temperate or cold	
Nahanni, Canada	12	61–62°N	Dcf	–4.5	566

* Information given by the authors listed was supplemented from a variety of other sources.

† van de Graaff *et al.*, personal communication (see text).

‡ The position of the Carnarvon area during the early Permian period was estimated from diagrams contained in ref. 35.

primarily responsible. Deep labyrinths are restricted to massive carbonates of low porosity and primary permeability that are crossed by widely spaced, but vertically and horizontally extensive, fractures or fracture zones. There are very few penetrable bedding planes. In many cases the fault and joint networks are results of recent uplift and flexing, which also provided the necessary local relief. Limestones and dolomites suitable for deep labyrinth karst development are comparatively uncommon explaining why this style is not more frequently encountered. Solutional labyrinths of much smaller scale, confined to the uppermost few metres of rock, are more numerous and are termed 'karrenfeld' or 'limestone pavements'^{42,43}.

Although certain broad influences of zonal climate on karst morphology cannot be denied, characteristics of the host carbonate shape the labyrinth karst style. Given an equal distribution of suitable rocks it is to be expected that there will be a greater frequency of this style in the tropics where karst evolution has not been interrupted by glacial events. Tower karst can no longer be regarded as specific to humid tropical climates for it can develop well in cold environments. Some cases where extra-tropical tower karst has been interpreted in terms of tropical palaeoclimatic conditions may require re-evaluation. If the distribution of tower karst is not dependent on climate one of two fundamental pieces of evidence that uphold the morphoclimatic approach to karst geomorphology is no longer valid. In future, more emphasis should be given to structural and lithological properties of the host soluble rock.

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Temperature-sensitive mutant of avian erythroblastosis virus suggests a block of differentiation as mechanism of leukaemogenesis

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A temperature sensitive mutant has been isolated for the first time from a replication defective acute leukaemia virus, AEV. In vivo, at 41 °C, the mutant shows a reduced leukaemogenic potential. In vitro, in erythroblasts transformed at 35 °C, haemoglobin synthesis can be induced by a shift to 41 °C. This indicates that the continuous expression of a viral gene product is necessary to maintain the undifferentiated state of the virus-transformed leukaemia cells.

THE development of *in vitro* transformation techniques for replication-defective leukaemia viruses in cultures of haematopoietic cells (see ref. 1 for review) introduced a new approach for the study of the mechanism of virus-induced leukaemogenesis. Results obtained mainly with avian erythroblastosis virus (AEV), myeloblastosis virus (AMV) and myelocytomatosis

virus strain MC29 and with the murine Friend and Abelson leukaemia viruses showed that these viruses selectively transform haematopoietic cells of different lineages of differentiation in a highly selective manner¹. Two important questions were raised by these studies. First, is the leukaemic transformation effected by these viruses accompanied by a block of differentiation in their target cells? And second, is a viral gene product necessary to maintain the leukaemic state of the infected cell?

To answer these questions we decided to search for temperature-sensitive mutants of AEV. This virus induces an acute erythroblastosis within 1–2 weeks post-infection if injected intravenously, and slowly developing sarcomas if injected intramuscularly². After infection of bone marrow cells in culture it induces the formation of rapidly proliferating erythroblast foci or colonies^{3–5}. It also induces a transformation of chicken embryo fibroblast cultures^{4,6}. However, AEV does not possess the *src* gene, the gene responsible for transformation of fibroblasts by RSV⁷.

This article reports the isolation of the first temperature-sensitive mutant of AEV and partially answers the questions posed above.

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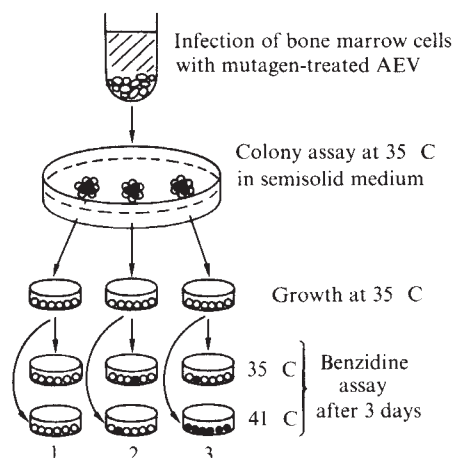


Fig. 1 Schematic representation of the procedure used to isolate temperature-sensitive mutants of AEV. The AEV used was a RAV-2 pseudotype of AEV-ES4 obtained as described earlier⁴. The virus was treated by mutagen by adding $5 \mu\text{g ml}^{-1}$ 5-azacytidine (Calbiochem) to 45×10^6 erythroblasts producing AEV in 10 ml of growth medium⁴. After 18 h at 37°C the erythroblasts were removed by low-speed centrifugation and the supernatant centrifuged at $70,000g$ for 30 min to pellet the virus. The pellet suspended in 5 ml growth medium was used to infect fresh bone marrow cells which were seeded at 10×10^6 cells per 35-mm dish in methylcellulose-containing medium (ref. 5, and in preparation). After 8–12 days of incubation at 35°C erythroblast colonies were isolated with a drawn out Pasteur pipette, transferred into the wells (15 mm) of a multidose tissue culture tray each containing 0.5 ml growth medium and propagated at 35°C until 10^5 – 10^6 cells were obtained from each colony. The erythroblast clones were then suspended in 1 ml growth medium, and divided equally into the respective wells of two new trays. One tray was shifted to 41°C , the other was kept at 35°C . After 3 days of incubation at the respective temperatures all cultures were stained with benzidine by the procedure of Orkin *et al.*⁸ to determine the proportion of haemoglobin-positive (B^+) cells. In the scheme, B^+ cells are drawn as closed circles, B^- cells as open circles. In the example shown, clone 3 behaves as expected from an erythroblast clone infected with a temperature-sensitive mutant, that is, B^- at 35°C and B^+ at 41°C ; clones 1 and 2 behave like wild-type-infected cells.

Isolation of erythroblast clones with temperature-sensitive properties

Initial attempts to isolate *ts* mutants of AEV using the fibroblast focus assay⁴ failed to yield the desired mutants (B. Royer-Pokora and T.G., unpublished). With the development of a new transformation assay of bone marrow cells which allows the isolation of clones of transformed cells characterised as immature erythroid cells (erythroblasts) by various differentiation markers and derived from the infection with a single AEV particle (ref. 5, and H.B. *et al.*, in preparation), an alternative approach became possible. The analysis of a large number of individual erythroblast clones obtained with this technique revealed that different clones vary in their haemoglobin expression measured as the proportion of benzidine-positive (B^+) cells. Most of these clones contained less than 10% B^+ cells⁵; this proportion, however, could be greatly increased by the addition of butyric acid⁵. These and other findings not reported here led to the hypothesis that AEV induces a block in differentiation in its haematopoietic target cells. Consequently, if it were possible to inactivate the viral functions in the infected cell, it should be possible to induce a differentiation in the AEV-transformed erythroblasts. Based on this idea a strategy was developed for isolating temperature-sensitive mutants of AEV which is outlined in Fig. 1. Erythroblast colonies were produced by infecting bone marrow cultures with mutagen-treated AEV at 35°C and seeding them into semisolid medium. Cells obtained from individual colonies were propagated at

35°C . Aliquots of these clones were then shifted to 41°C or kept at 35°C and assayed for the proportion of B^+ cells by benzidine staining 3 days later.

From 94 erythroblast clones derived from mutagen-treated virus and assayed in this way, three clones (numbers 3, 34 and 43) were temperature-sensitive for haemoglobin expression, containing more than 70% strongly B^+ cells at 41°C as compared to <10% at 35°C . In contrast, none of 26 colonies derived from untreated AEV showed this type of temperature sensitivity.

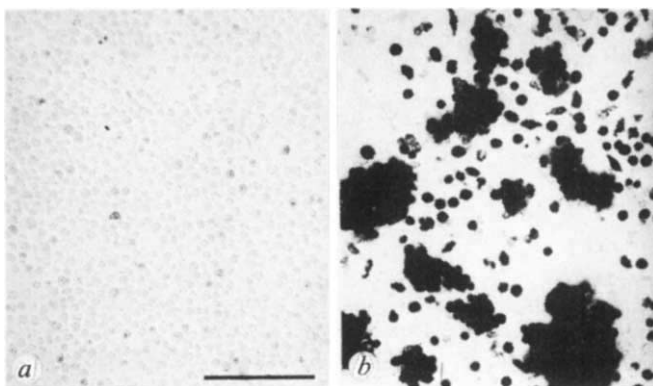
AEV from erythroblast clone 34 is temperature sensitive

To determine whether clones 34 and 43 produce virus capable of inducing erythroblast colonies temperature sensitive for haemoglobin expression (clone 3 was lost), cell-free supernatants from both clones were used to infect fresh bone marrow cultures at 35°C . In addition, bone marrow cells were infected with wild type (*wt*) AEV. Between 300 and 1,000 colonies per dish were obtained with the three inocula. Twenty colonies of each type were isolated at random and the 10 fastest growing clones were tested for temperature sensitivity. All of the 10 erythroblast clones derived from clone 34 virus (*ts34* AEV) could be induced to express haemoglobin, showing an increase from an average of about 13% B^+ cells at 35°C to about 76% at 41°C (Table 1, expt 1). In contrast, with the exception of clone 4, *wt* AEV-infected clones showed essentially identical, relatively low proportions of B^+ cells at both 35 and 41°C (Table 1, expt 1). The virus produced by clone 4 was characterised as *wt* AEV by its inability to induce temperature-sensitive erythroblast colonies (data not shown). The 10 clones induced by virus from clone 43 cells exhibited variable proportions of B^+ cells at 41°C , fluctuating between less than 1% to 59% or greater with an average of 32% (data not shown). It is remarkable that with *ts34* AEV, in general, those clones which already exhibited relatively high proportions of B^+ cells at 35°C also showed the highest proportions of B^+ cells after shift to 41°C . Photographs of *ts34* AEV-infected erythroblasts kept at 35°C and at 41°C and stained with benzidine are shown in Fig. 2.

The *ts* lesion is located in the defective, transforming component of *ts34* AEV

The above results indicate that the temperature sensitivity of haemoglobin expression in erythroblast clones 34 and possibly 43 was due to the infecting AEV/helper virus complex and not to a property inherent to the particular host cells. To determine whether the temperature sensitivity of *ts34* AEV was due to a mutation in AEV rather than in the helper virus (RAV-2),

Fig. 2 Effect of temperature on the expression of haemoglobin in *ts34* AEV-infected erythroblasts. Erythroblasts from a single clone were grown at 35°C and aliquots seeded at 35°C (a) and 41°C (b). Three days later the cells were stained with benzidine. The dark cells represent B^+ cells. Aggregation of cells at 41°C usually precedes the appearance of B^+ cells by 1–2 d. Scale bar, $100 \mu\text{m}$.



attempts were made to obtain nonproducer erythroblasts containing only the AEV genome. Erythroblast colonies were isolated from bone marrow suspension cultures infected with *ts34* AEV at low multiplicity. After propagation of the isolated colonies at 35 °C, the supernatants were assayed for reverse transcriptase activity (AEV-transformed nonproducer erythroblasts do not release reverse transcriptase-positive virus particles⁹) and for the production of infectious virus using the bone marrow colony assay to score for nonproducer erythroblast clones. Of eight clones tested, four were found to be nonproducer by both types of assay. All eight *ts34* AEV-infected erythroblast clones, regardless of whether or not they produced virus, were temperature sensitive for the expression of haemoglobin. This demonstrates that a lesion in AEV and not in the helper virus is responsible for the observed temperature-sensitive expression of haemoglobin in *ts34* AEV-infected cells. To further substantiate this conclusion, pseudotypes of *ts34* AEV were produced by superinfection of a *ts34* AEV nonproducer clone with standard helper viruses. Colonies induced by these pseudotypes were then tested for temperature

Quantitation of haemoglobin expression in *ts34* AEV-infected erythroblasts

Two types of assay were used to obtain quantitative estimates of haemoglobin expression after shift of *ts34* AEV- or *wt* AEV-infected erythroblast clones to 41 °C. First, cell extracts were reacted with benzidine and assayed photometrically; and second, a newly developed competition radioimmunoassay for chicken haemoglobin was used. For comparison, before extraction the same clones were subjected to the cytological benzidine assay. The results of these experiments are shown in Table 2. With the benzidine photometric assay a 10- to 15-fold increase was observed in the *ts*-clones 3 days after shift to 41 °C as compared to a 2 to 4-fold increase in the *wt* clones. When the same extracts were tested for haemoglobin by competition radioimmunoassay, a 5 to 7-fold increase was observed in the *ts* clones whereas no or only a slight increase (1.5–2.5-fold) was observed in the *wt* clones.

The smaller increase in haemoglobin levels of the *ts* cells observed by these techniques as compared to the cytological

Table 1 Assay of temperature sensitivity of erythroblasts infected with various pseudotypes of *ts34* AEV and *wt* AEV

Expt	Virus used for infection	Incubation temperature (°C)	Clone no. (% of B ⁺ cells)									
			1	2	3	4	5	6	7	8	9	10
Expt 1	<i>ts34</i> AEV	41°	65	59	59	60	98	91	91	95	61	87
		35°	0	1	4	2	33	33	17	37	2	18
	<i>wt</i> AEV	41°	0	0	0	49	0	16	0	0	13	0
		35°	0	0	0	26	0	31	0	0	13	0
Expt 2	<i>ts34</i> AEV (RAV-2)	41°	35	60	55	40	90	75	25	60	—	—
		35°	1	1	4	1	22	5	0	4	—	—
	<i>ts34</i> AEV (SR-RSV-D)	41°	55	75	—	—	—	—	—	—	—	—
		35°	5	8	—	—	—	—	—	—	—	—
	<i>ts34</i> AEV(<i>wt</i> AEV)	41°	85	55	0	60	1	2	60	—	—	—
		35°	16	17	0	0	0	0	1	—	—	—
	<i>wt</i> AEV(RAV-2 <i>ts34</i>)	41°	0	1	1	25	0	0	5	2	—	—
		35°	0	1	0	17	0	0	2	0	—	—
		41°	—	—	—	—	—	—	—	—	—	—
		35°	—	—	—	—	—	—	—	—	—	—

Pseudotypes of *ts34* AEV were obtained by inoculating 5×10^5 cells of a clone of *ts34* AEV nonproducer erythroblasts at 35 °C with about 10^5 infectious units of RAV-2, SR-RSV-D or AEV (RAV-2). The *wt* AEV (RAV-2 *ts34*) pseudotype was obtained by superinfecting a clone of *wt* AEV nonproducer erythroblasts⁹ with RAV-2 which had been plaque purified¹⁰ from the original stock of *ts34* AEV. Virus collected 5 days post-infection (p.i.) was used to infect bone marrow cultures at 35 °C. Twenty to 80 colonies were obtained in the cultures infected with the pseudotypes, no colonies were obtained with the supernatant of nonproducer erythroblasts without added helper virus. Colonies were isolated, propagated and tested as described in Fig. 1. In the cultures stained with benzidine a minimum of 250 cells were evaluated. In expt 1, erythroblasts were shifted 17 days p.i. (that is, at about 15–20 population doublings), in expt 2, 24 days p.i. (~22–27 population doublings).

sensitivity. Regardless of whether RAV-2, SR-RSV-D (Table 1, expt 2) or RAV-1 (data not shown) were used as helper virus, all pseudotypes of *ts34* AEV still induced temperature-sensitive erythroblast colonies. In a similar experiment using *wt* AEV (which contains RAV-2 as helper virus) for superinfection of *ts34* AEV nonproducer erythroblasts, the resulting virus mixture induced both wild-type (3 out of 7) and temperature-sensitive (4 out of 7) colonies (Table 1, expt 2). This indicates that *ts34* AEV-infected erythroblasts can be superinfected with *wt* AEV.

An independent line of evidence shows that the original helper virus of *ts34* AEV is not responsible for the observed temperature-sensitive properties of the latter: a pseudotype consisting of *wt* AEV and the helper virus isolated from the original *ts34* AEV virus stock failed to cause colonies which were temperature sensitive for the expression of haemoglobin (Table 1, expt 2).

These results suggest that a gene product of AEV blocks the accumulation of haemoglobin, one of the major events during normal erythroid differentiation. In the following, this notion is substantiated by quantitative data on haemoglobin expression and by kinetic studies.

assay probably reflects a threshold effect of the latter method. The expression of haemoglobin in the induced erythroblasts is still far less complete than in fully differentiated cells: depending on technique used, only 2 to 8% of the haemoglobin levels of erythrocytes is expressed in the *ts34* AEV erythroblasts 3 days after shift. Assuming that the benzidine technique measures the levels of protein-bound and free haem and that the radioimmunoassay detects the levels of haemoglobin as well as free globin, the data in Table 2 suggest that there is a more pronounced or more rapid induction of haem synthesis as compared to globin synthesis in these cells in response to the shift. In addition, our data indicate that in AEV-infected erythroblasts at the 'undifferentiated' stage there is an excess of globin over haem, assuming that equimolar amounts of haem and globin are present in mature erythrocytes.

Kinetics and reversibility of haemoglobin expression

To study the time course of temperature-dependent induction of haemoglobin synthesis, *ts34* AEV-infected erythroblasts were shifted from 35 to 41 °C and aliquots were stained with ben-

Table 2 Haemoglobin expression in *ts34* AEV-infected erythroblasts

Cells tested		% Benzidine positive cells	Hb expression in cell extracts:		
			Benzidine staining	Competition radioimmunoassay	
			A_{515} per mg protein	μg haemoglobin per mg protein	
			41°/35°	41°/35°	
<i>ts34</i> AEV-erythroblasts	35 °C	0, 1.4, 1.7	0.8, 1.0, 0.8	5.4, 5.4, 6.2	
	41 °C	73, 68, 87	8.2, 9.2, 10.6	38, 32, 32	7.1, 5.8, 5.1
<i>wt</i> AEV-erythroblasts	35 °C	0, 2.4, 1.6	<0.2, 1.2, 0.6	3.8, 16.6, 4.4	
	41 °C	0.1, 7.2, 3.9	1.6, 2.2, 2.0	7.2, 12.4, 12.8	1.9, 0.8, 2.8
Chicken erythrocytes		100, 100	446, 394	474, 528	—
Chicken fibroblasts, macrophages		0, 0	<0.2, <0.2	<0.5, <0.5	—

Three clones each of *ts34* AEV- and *wt* AEV-‘transformed’ erythroblasts grown at 35 °C for ~25 population doublings were seeded at 12 to 30×10^6 cells in 10 ml growth medium in two 100-mm dishes. One of the dishes was kept at 35 °C, the other was shifted to 41 °C. After 3 days, small aliquots of each clone were separated for protein determination¹² and for staining with benzidine⁸ to reveal the proportion of B⁺ cells and with Trypan blue to reveal the proportion of dead cells. Cell extracts were prepared essentially according to the procedure of Conscience *et al.*¹¹. Of these extracts, 5–50 μl were used for benzidine staining using the procedure of Crosby *et al.*¹³ and for the competition radioimmunoassay as outlined below. The values shown are the data obtained with the three different clones whereby position 1 always corresponds to clone 1, position 2 to clone 2 and position 3 to clone 3. The values shown were corrected for the proportion of dead cells. Competition radioimmunoassay: chicken haemoglobin was prepared from washed erythrocytes of adult chickens as described by Bruns and Ingram¹⁴. This preparation (~90% pure by SDS-polyacrylamide gel electrophoresis and staining with Coomassie blue) was concentrated to 11 mg ml⁻¹ by ultrafiltration and dialysed against 0.1 M borate buffer pH 8.5. Haemoglobin (30–60 μg) was then labelled with 0.5–1 mCi [¹²⁵I]Bolton-Hunter reagent (Amersham, 1,700 Ci mmol⁻¹) as described¹⁵. Labelled protein was separated from unreacted iodinated ester and other by-products on a Sephadex G75 superfine (Pharmacia) column (0.5 $\times$ 10 cm) equilibrated with 0.1 M borate buffer pH 8.5 containing 0.25% gelatine. After the addition of 1% Triton X-100 the labelled haemoglobin was stored in aliquots at -70 °C for up to 6 weeks. Radioactivity of the labelled product was between 3 and 6×10^6 c.p.m. per μg protein and of this >90% was in haemoglobin as judged by SDS-polyacrylamide gel electrophoresis and autoradiography. More than 60% of the labelled product was precipitable with an excess of antibody. The reaction mixture for competition radioimmunoassay contained between 50,000 and 60,000 c.p.m. (± 10 –20 ng) of [¹²⁵I]-haemoglobin, 2.5 μg of rabbit immunoglobulin directed against chicken haemoglobin (Cappel Laboratories) dissolved in 10 μl RIA-buffer (0.01 M Tris pH 7.0; 0.1 M NaCl and 1% Triton X-100), 5–50 μl cell extract to be assayed for haemoglobin and RIA-buffer up to a final volume of 400 μl . After incubation for 2 h at 37 °C and 2 h at 4 °C, 10 μl of a 10% (v/v) *Staphylococcus aureus* suspension prepared according to Kessler¹⁶ were added and the mixture incubated for another 30 min at 37 °C. The reaction mixture was washed by centrifugation through 500 μl of 10% sucrose in RIA buffer and the pellet was resuspended in 600 μl RIA buffer followed by another centrifugation. The radioactivity of the sediment was then determined in a gamma counter. In a typical calibration curve, using a standard haemoglobin preparation, 2 ng of cold haemoglobin resulted in 20% competition; 9 ng in 50% competition and 60 ng in 90% competition.

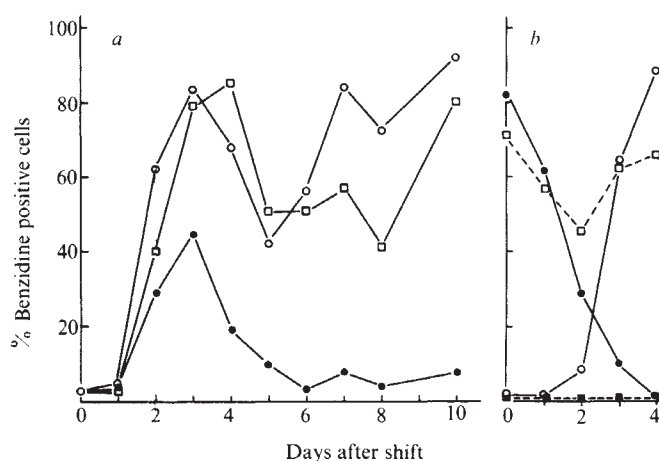
zidine at daily intervals thereafter. As can be seen from Fig. 3, the proportion of B⁺ cells reached a maximum 3 to 4 days after shift. Thereafter, in most experiments, a slight temporary decline was seen which remains unexplained so far. No significant difference in the kinetics was observed in the absence or presence of helper virus (Fig. 3a). After superinfection of *ts34* AEV nonproducer erythroblasts with *wt* AEV at the time of shift to 41 °C, only a transient expression of haemoglobin within the first 2 days was observed. After 4 to 6 days, haemoglobin expression was again suppressed almost to control levels (Fig. 3a). As shown in Fig. 3b, the increased expression of haemoglobin induced by shift to 41 °C could also be reversed by a subsequent downshift of the *ts34* AEV erythroblasts to 35 °C, exhibiting a similar time course as seen in the upshift experiments.

Leukaemogenicity of *ts34* AEV

If *ts34* AEV does in fact cause a block of differentiation in its target cells at 35 but not at 41 °C, and if this differentiation block is correlated to or even causative of leukaemic transformation, then *ts34* AEV should also exhibit a decreased *in vitro* transforming ability at 41 °C as well as a reduced leukaemogenicity *in vivo* (the body temperature of the chicken exceeds 41 °C).

Assay of *ts34* AEV in bone marrow cells using the methylcellulose colony assay yielded comparable numbers of colonies at 35 and 37 °C (580 and 480) but no colonies at 39 °C. In contrast, however, the number of colonies obtained with *wt* AEV at 39 °C (330) was only slightly lower than at 35 and 37 °C (420 and 370). At 41 °C, no colonies were obtained with either *ts34* AEV or with *wt* AEV, probably reflecting a general inability of normal and transformed chick bone marrow cells to

Fig. 3 Kinetics of haemoglobin expression and effect of superinfection with *wt* AEV of *ts34* AEV-infected erythroblasts. Aliquots were stained with benzidine at different times after shift. In (a), cells from *ts34* AEV nonproducer erythroblasts (clone A6) were shifted from 35 to 41 °C without superinfection (○), after superinfection with RAV-2 (□) or after superinfection with *wt* AEV (RAV-2) at the day of shift (●). In (b), *ts34* AEV cells from a virus-producing erythroblast clone (C2) were shifted from 35 to 41 °C (○) or from 41 to 35 °C (●). As controls, cells were kept at 35 °C (■) or at 41 °C (□). The 41 °C control cells had been shifted from 35 to 41 °C 3 days before the experiment. In all cases, cells were kept at about 1.5×10^6 per ml in growth medium during the experimental period.



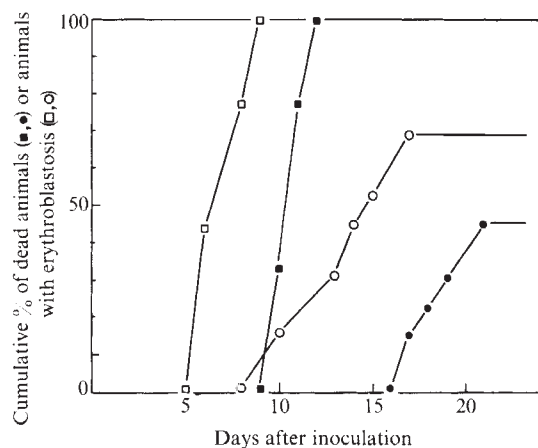


Fig. 4 Leukaemogenicity of *ts34* AEV. Three groups of 8-day old chicks were injected i.v. with 0.1 ml of growth medium containing either 1,000 colony-forming units of cell-free *ts34* AEV (RAV-2) (13 chicks); 10 colony-forming units of cell-free *wt* AEV (RAV-2) (9 chicks); or growth medium without virus (9 chicks). All animals were observed for the appearance of erythroblastosis by preparing blood smears at 2–3-day intervals and for death over a period of 3 months. Chicks with *ts34* AEV, circles; with *wt* AEV, squares. Solid symbols, cumulative % death; open symbols, % erythroblastosis.

form colonies in semisolid medium at 41 °C (unpublished observations).

The data in Fig. 4 show that *ts34* AEV is also less leukaemogenic than *wt* AEV. This was not only manifested in the decreased incidence of animals which died of leukaemia (46% as compared to 100% in control animals injected with 100 times fewer colony-forming units of *wt* AEV), but also in a delay of about 1 week in the onset of the disease and in the death of the animals. Interestingly, erythroblastosis spontaneously regressed in three of the mutant-infected chicks. We have never observed such a regression in birds infected with *wt* AEV. Two of the regressors were challenged with about 1,000 colony-forming units of *wt* AEV at 3 months of age and both died of erythroblastosis within 3 weeks post-infection. This indicates that the animals were still fully susceptible to the leukaemia and that the regression observed was probably not due to immunological defense mechanisms.

To determine whether or not the erythroblasts in the *ts34* AEV-infected animals were temperature-sensitive for haemoglobin expression, peripheral blood cells obtained from leukaemic animals were seeded at various concentrations into 15-mm tissue culture wells containing growth medium and incubated at 35 and 41 °C. Six days later foci of growing erythroblasts had developed at both temperatures and these were stained with benzidine *in situ*. The incidence of predominantly B⁺ foci was 2.5% at 35 °C (38 colonies evaluated) and 93% at 41 °C (71 colonies evaluated). In addition, B⁺ foci at 41 °C were somewhat smaller than the foci obtained at 35 °C. Similar results were also obtained with erythroblasts from two further *ts34* AEV-infected chicks, whereas only 7% of the foci at 41 °C (42 colonies evaluated) from a *wt* AEV-infected animals were B⁺.

These results indicate that *ts34* AEV is less leukaemogenic than parental AEV and that the majority of leukaemic erythroblasts from the peripheral blood of mutant-infected chicks still exhibit temperature-sensitive properties.

Discussion

The results presented here show that in erythroblasts transformed by *ts34* AEV the expression of haemoglobin can be induced by raising the temperature from 35 to 41 °C. Together with our finding that the target cell of AEV is an immature erythroid cell (ref. 5 and unpublished results) and the observation that *ts34* AEV showed a decreased leukaemogenicity *in*

vivo, this indicates that AEV induces a block of differentiation in its erythroid target cells and that this block may play a crucial part in virus-induced leukaemogenesis. Preliminary findings showed that *ts34* AEV is still capable of transforming fibroblasts at 41 °C. ARSO, AEV does not possess the *src* gene responsible for sarcoma formation by avian sarcoma viruses⁷. This raises the possibility that virus-induced leukaemogenesis is a process different from fibroblast transformation by avian sarcoma viruses. In this regard it will be interesting to determine whether transformation parameters as defined in the fibroblast system⁶ are also expressed in *ts34* AEV-transformed erythroblasts at 35 °C but not at 41 °C.

Our results are not compatible with the notion that the integration of AEV at a specific site into its host cells is sufficient to induce a leukaemic transformation. Instead, they indicate that an AEV-coded gene product has to be continuously expressed to maintain the block in differentiation and therefore also the leukaemic state of the cell. Whether this gene product is a protein and whether a host function has to be expressed in addition to it remains to be determined. A candidate for an AEV-coded protein responsible for leukaemic transformation is a 75,000 molecular weight polypeptide synthesised in AEV-nonproducer erythroblasts and fibroblasts⁹. This protein consists of part of the *gag* gene of the virus and an unknown portion with a MW of at least 55,000 (ref. 9).

In experiments in which *ts*-mutant-infected erythroblast clones were tested at rather high passages (after ~25 population doublings) only a fraction of the cells from certain clones were inducible and also had an increased tendency to lyse after shift to 41 °C (see Table 1, expt 2 and unpublished observations). Such 'aged' clones infected with *wt* AEV showed also a slight increase in haemoglobin expression after shift to 41 °C (see Table 1, expt 2 and Table 2) and we believe that this reflects a property of the host cells and not of the virus.

The *in vivo* studies with *ts34* AEV clearly show that it is less leukaemogenic than the parental *wt* AEV. Its leakiness is probably due both to its residual ability to block differentiation at 41 °C as suggested by the low levels of haemoglobin expression in some *ts34* AEV-erythroblast clones *in vitro* as well as to the selection of wild-type revertants *in vivo*. A revertant of *ts34* AEV has indeed been isolated from erythroblasts obtained from a *ts34* AEV-infected animal (unpublished results). Interestingly, the well characterised *ts68* mutant of Rous sarcoma virus appears to have an even higher residual oncogenic potential than *ts34* AEV¹⁷.

The observed 'differentiation' of *ts34* AEV-erythroblasts in response to upshift for 3 days was clearly incomplete in that the highest haemoglobin levels expressed in these cells did not exceed 8% of the amount present in mature erythrocytes. This raises the question as to whether the observed effects truly represent a step within normal erythroid differentiation. However, the conditions for induction tested may not yet have been optimal. Thus, in preliminary experiments in which *ts34* AEV erythroblasts were shifted for 8 days to 41 °C, levels of haemoglobin corresponding to about 25% of the amount present in erythrocytes were found. In addition, it might be possible to improve the physiological conditions required for optimal induction and to isolate less leaky mutants of AEV.

Obviously, the exact nature of the AEV-induced block in differentiation needs further clarification such as by studying the influence of metabolic inhibitors on the changes in haemoglobin synthesis and by testing additional parameters of erythroid differentiation. However, it seems already safe to say that chick erythroblasts infected with *ts* mutants of AEV provide a unique new system to study erythroid differentiation *in vitro*, and this can be exploited in a similar way as the induction of differentiation of mouse erythroleukaemia cell lines infected with Friend leukaemia virus by treatment with chemicals¹⁸. In addition, the AEV system has the advantage over the Friend system that possible side effects of chemicals can be avoided and that different diploid clones of 'transformed' erythroblasts can be analysed shortly after infection.

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A two-step fibrinogen–fibrin transition in blood coagulation

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The kinetics of the thrombin-catalysed release of fibrinopeptides A and B from human fibrinogen have been investigated and a mechanism correlating the release of fibrinopeptides to fibrin formation is presented. The sequential release of fibrinopeptides results in sequential activation of two sets of polymerisation sites.

WHEN thrombin catalyses the release of fibrinopeptides A and B (FPA and FPB) from mammalian fibrinogen, FPA must appear^{1–5}, followed, after a lag phase, by FPB, for polymerisation to occur. Preferential release of FPB by an enzyme in copperhead snake venom (*Agkistrodon contortrix*) does not result in normal polymer formation unless FPA is also removed^{6,7}. Light-scattering studies^{8,9} have demonstrated two types of initial polymerisation geometry of thrombin-activated fibrinogen. At near-neutral pH the geometry is predominantly end-to-end followed by lateral aggregation⁸. Fibrin formed when FPA is released has a lower mass-to-length ratio than that formed after release of both FPA and FPB¹⁰. Thus, it has been proposed that two sets of polymerisation sites are involved in fibrin formation^{10–12}: one set, A:a, is activated on release of FPA and results in end-to-end polymerisation; the other, B:b, is activated on release of FPB and results in lateral aggregation. Shen *et al.*¹⁶ speculated that the lateral aggregation following release of FPB may be of particular importance for rapid growth of the fibrin fibre. There is direct experimental evidence for at least one set of binding sites, probably the proposed A:a site^{13–15}. Here we report evidence for the existence of the B:b binding sites and that interaction at these sites is responsible for the enhancement of release of FPB observed during fibrin formation¹⁷.

Fibrin formation with and without Ca²⁺

Thrombin cleaves the bonds Aα16Arg–17Gly and Bβ14Arg–15Gly in human fibrinogen. A third bond, Aα19Arg–20Val, is cleaved more slowly¹⁸. Removal of FPA and FPB uncovers Gly as N-terminal residue on both α- and β-chains, but the γ-chain terminus is Tyr. We used N-terminal analysis to estimate the release of FPA and FPB and the cleavage of the third bond using bovine thrombin. These results were corroborated by radioimmunoassay (RIA) for FPA and FPB^{4,5}. Polymerisation was measured by ultraviolet absorption at 450 nm. Control experiments with human thrombin (3,000 NIH units per mg) showed no significant difference. Figure 1a shows the results of N-terminal analysis on thrombin digests of human fibrinogen. The

lower part of this curve corresponds to release of FPA³ which is followed by gel formation, and the upper part is due to the release of FPB³. There was no significant change in the amount of N-terminal valine throughout the digestion.

In similar experiments, FPA and FPB were determined by RIA (Fig. 2a–c). As in previous studies^{2–5}, at all pH values FPA was released first, followed by FPB after a lag phase during which polymerisation began. Rate of release, especially of FPB, was greater at higher pH and the lag phase became shorter. This effect was more evident when lower concentrations of thrombin were used. The gel point occurred when no (pH 6.3) or little (pH 7.4 and 9.0) FPB had been released. At pH 9.0 the gel was transparent; below pH 8.4–8.5 it was opaque, in agreement with the findings of Ferry and Morrisson¹⁹.

Ca²⁺ binds to fibrinogen, shortening the clotting time²⁰, and chelating agents strongly inhibit fibrin formation^{21,22}. We found that the rate of release of the two peptides at different pH values was essentially unaffected by Ca²⁺ (20 mM) and the transition from an opaque to a transparent clot was not observed in the pH range studied.

Release when polymerisation is impaired

Because the release of FPB seemed to be greatly accelerated after the formation of a clot (Figs 1 and 2), we studied its release in systems where polymerisation was impaired. Fibrinogen was digested in 2 M urea and proteolysis was assessed by N-terminal analyses (Fig. 1b). The ratio of Gly:Tyr of 1:1 suggested that only one fibrinopeptide was released when botroxobin fibrin, which is devoid of FPA³, was digested with thrombin (Fig. 1c), and the slow appearance of N-terminal Gly indicated the slow release of FPB. When a non-polymerising N-terminal fragment of fibrinogen (N-DSK)²³ was used as substrate in the absence of urea (Fig. 1d), again only one fibrinopeptide seemed to be released. When N-DSK from botroxobin fibrin was digested, in the absence of urea, the result was similar to that in Fig. 1c.

Digestion of fibrinogen (60 μM) with thrombin in 2 M urea was repeated and the release of FPA and FPB was followed by RIA (Fig. 3a). FPA was released completely but only small amounts of FPB were released and no absorbance increase at 450 nm was observed, indicating that polymerisation had not occurred. When the digest was diluted six times with buffer (pH 7.4) a polymer was formed almost instantaneously and FPB was released rapidly (Fig. 3b). To show that this was not a consequence of changes in fibrinogen or thrombin, caused by urea or on dilution of the urea solution, fibrinogen (60 μM) in 2 M urea was kept for 1 h at 37°C and then diluted with buffer containing thrombin. On dilution, polymerisation occurred and

the sequential release of FPA and FPB was the same as with fibrinogen not pretreated with urea. In a second control experiment, thrombin (0.95 NIH units ml^{-1}) was incubated in 2 M urea for 1 h and then diluted in buffered fibrinogen to give a solution which was 0.33 M in urea and 7 μM in fibrinogen (pH 7.4), and containing 0.16 NIH units of thrombin per ml. Polymerisation occurred in this system and the pattern of release of FPA and FPB was the same as for native fibrinogen at similar thrombin concentrations. Other control experiments were carried out with derivatives of fibrinogen having no capacity to polymerise; botroxobin-treated N-DSK (devoid of FPA but containing FPB) was digested with thrombin in 2 M urea (Fig. 3c). No substantial amounts of FPB were released, but on dilution, it was released slowly (Fig. 3d). FPB was released from polymerising fibrin (Fig. 3b) much faster than from N-DSK in

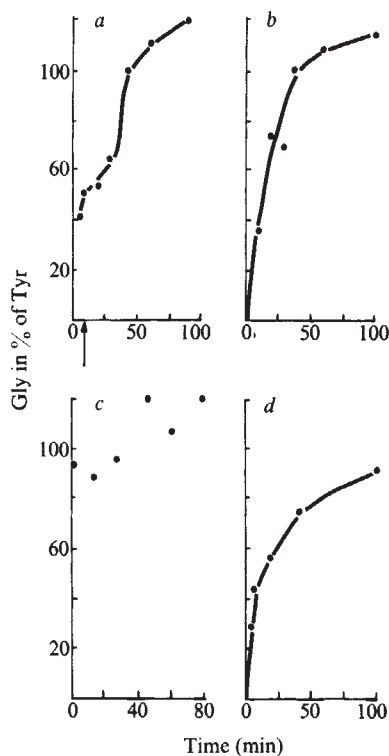


Fig. 1 Appearance of N-terminal glycine during digestion with thrombin of fibrinogen and derivatives of fibrinogen. Human fibrinogen (95% clottable), fibrinogen and fibrin N-DSK preparations were as described previously^{23,35}. Bovine thrombin, 1,800 NIH units per mg, was purified as described elsewhere³⁵. Botroxobin fibrin was prepared by adding 10 μl of an aqueous solution of botroxobin (from *Botroxobin B marajonesis*, 2,400 BU per mg, Pentapharm, 0.5 mg ml^{-1}) to 6 ml of 1% solution of fibrinogen in 0.15 M NaCl. The solution was incubated at 37 °C for 4 h. The fibrin clot was dissolved in 6 ml 4 M urea and dialysed at +4 °C against 25 mM Tris-HCl, pH 7.2, 2 M in urea and 0.125 M in NaCl. The fibrin solution was diluted to 20 μM with the same buffer and stored at -20 °C in 2 ml portions. Substrates (20 μM) were digested with thrombin at 37 °C in 25 mM Tris-HCl, 0.125 M NaCl, pH 7.2 (where indicated, the medium was 2 M in urea). The procedures used in following the hydrolysis have been described in detail³⁵, 'method A' being used for substrates *b* and *c* and 'method B' for substrate *d*; for substrate *a* the procedures were modified because fibrin clot formation prevented removal of a representative aliquot of the digest. This problem was circumvented by carrying out the digestion in eight tubes, one tube for each assay time, and thereafter carrying out N-terminal analysis on the contents of each tube according to method A³⁵. *a*, Fibrinogen, thrombin = 0.18 NIH units ml^{-1} . Arrow indicates the time of formation of a solid gel; *b*, fibrinogen in 2 M urea, thrombin = 0.82 NIH units ml^{-1} ; *c*, botroxobin fibrin in 2 M urea, thrombin = 4.0 NIH units ml^{-1} ; *d*, fibrinogen N-DSK, thrombin = 0.18 NIH units ml^{-1} .

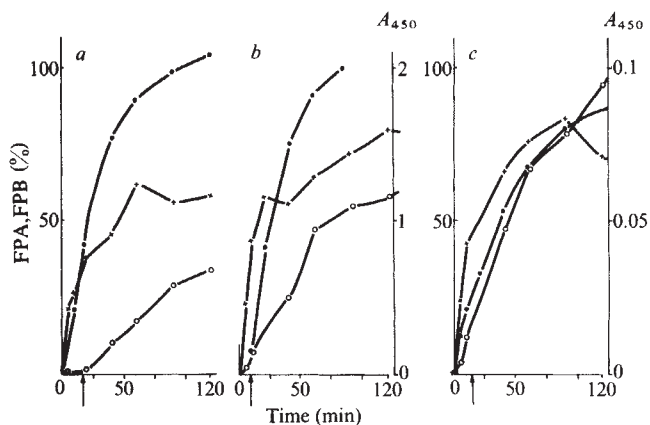


Fig. 2 Thrombin-induced release of FPA and FPB from fibrinogen as determined by radioimmunoassay (RIA) technique. Human fibrinogen (93–100% coagulability) was diluted in Tris-imidazole-HCl buffer⁸ (pH 6.35, 7.4 or 9.0) to give a final solution 17 mM in Tris and imidazole, ionic strength 0.15 and protein concentration 8–10 μM . Fibrinopeptide assay: the fibrinogen solutions were prewarmed at 37 °C. Aliquots of 200 μl were placed in eight siliconised tubes. Bovine thrombin (specific activity 200 NIH units per mg) was added to a final concentration of 0.025 or 0.05 NIH units ml^{-1} and incubated at 37 °C. The digestion was interrupted by adding 0.1–0.2 AT units of hirudin (Pentapharm) at different incubation times. Urea (200 μl of 8 M) was added to dissolve clots formed or, in some cases, the clots were removed before analysis. Radioimmunoassay (RIA) of FPA and FPB was carried out as described elsewhere^{4,5}. Polymerisation: As each of the above samples was taken for RIA determination, a separate prewarmed sample of 1 ml was put into a quartz cuvette and thrombin was immediately added to the same concentration as above. Optical density at 450 nm against time was monitored in an Acta C III spectrophotometer (LKB-Beckman). The transparent type of clot (see Fig. 2c) was not formed in the presence of Ca^{2+} . The time required for formation of a solid gel is indicated by arrows. The latter determinations were carried out in separate tubes. *a*, pH 6.35; *b*, pH 7.4; *c*, pH 9.0. ●, A_{450} ; ×, FPA; ○, FPB.

the same conditions. We believe that the polymer formed on dilution of thrombin-treated fibrinogen in urea solution represents a physiological fibrin structure, because it subsequently cross-linked in the presence of factor XIII_a to give a polyacrylamide gel pattern (Fig. 3, insert) indistinguishable from that produced when fibrinogen, in the absence of urea, was clotted with thrombin in the presence of factor XIII_a and Ca^{2+} .

Another derivative of fibrinogen with impaired gel-forming capacity is obtained by photooxidation²⁴. When photooxidised fibrinogen was exposed to thrombin, FPA only was released rapidly. When this substrate was digested with thrombin in 2 M urea, FPA was released fast and FPB slowly. On dilution of this system, the release of FPB was only slightly increased.

We next investigated congenitally dysfunctional fibrinogen from individuals homozygous and heterozygous for dysfibrinogenemia Detroit, in which fibrin formation is delayed^{25–27}. In this condition FPA is released at an almost normal rate, but the release of FPB is slow. When normal and affected fibrinogen were exposed to thrombin (Fig. 4a–c) the release of FPA was always fast. In homozygous fibrinogen Detroit no polymer formation was observed during FPA release and the initial release of FPB was slow (Fig. 4c). After prolonged incubation, polymerisation occurred and the release of FPB was accelerated concomitantly (Fig. 4c). Heterozygous fibrinogen Detroit (Fig. 4b) gave the same patterns as normal fibrinogen (Fig. 4a), as expected, because the heterozygous fibrinogen contains about 50% 'normal' fibrinogen^{25,27}, providing an initial concentration of 'normal' A α -chain which is almost twice the K_m for thrombin-catalysed cleavage of this domain in fibrinogen²⁸.

To demonstrate the causal link between delayed polymerisation and the release of FPB, fibrinogen Detroit was

digested with botroxobin (Fig. 5b). FPA was released but polymerisation and release of FPB occurred only when thrombin was added (Fig. 5c).

Sequence of events in formation of fibrin

It is thought that one binding domain (A) becomes active after release of FPA and is located in the N-DSK region of fibrinogen¹³⁻¹⁵. This domain interacts with a C-terminal domain (a) located in a plasmin fragment of fibrinogen, fragment D. In

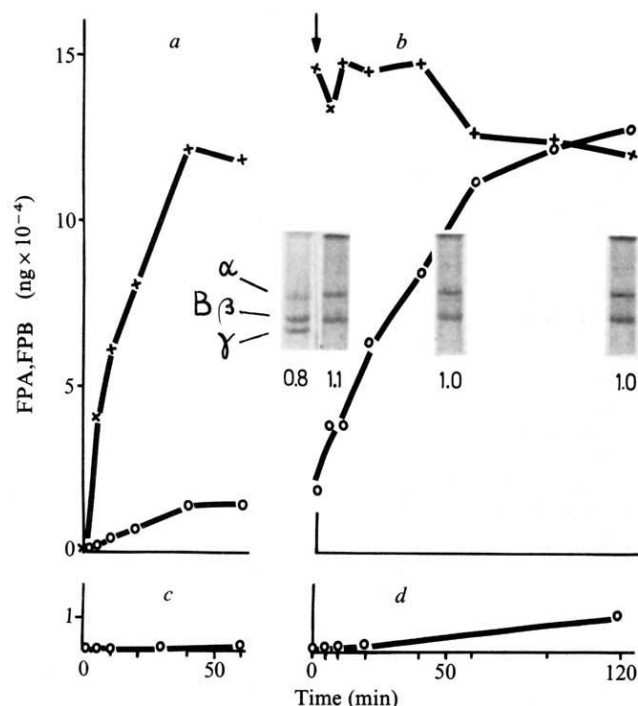


Fig. 3 Thrombin-induced release of FPA and FPB from fibrinogen and N-DSK in urea. *a*, Fibrinogen (see legend to Fig. 2) was reconstituted in 2 M urea to give a solution 0.3 M in NaCl and 60 μ M in protein. This solution (pH ~7) was incubated at 37°C with bovine thrombin (200 units per mg) at a concentration of 0.8 NIH units per ml. After various time intervals, aliquots (200 μ l) were taken and thrombin quenched with hirudin (1.0 AT units). RIA was carried out as described in the legend to Fig. 2. *b*, After digestion for 1 h the bulk of the digest was used as follows: 50- μ l aliquots were transferred to a series of siliconised glass tubes. A sixfold dilution was obtained by adding 250 μ l of 17 mM Tris-imidazole-HCl buffer, pH 7.4, containing 24 mM calcium acetate, 2.5 mg factor XIII (Behringwerke) and 1 μ l of Trasylol (Bayer), containing 1,500 KIE per ml, and digests were incubated at 37°C for various times. The reaction was interrupted by adding hirudin (0.5 AT units). Clots (arrow) were formed after dilution and these were removed from one set of experiments for SDS-gel electrophoresis (see inserts). The gel furthest to the left shows that the digest, before dilution, contains non-cross-linked α , B β and γ -chains. In the other gels poly α (at the top) and γ - γ dimers (just above α chain) are seen. The clots from another set were dissolved (8 M urea in 0.2 M NaOH) and protein determined at 282 nm using $\epsilon = 5.1 \times 10^{-5}$ (ref. 24). The amount (mg) of protein obtained is shown at the bottom of the SDS gels. FPA and FPB were determined on the clot supernatants using the RIA technique as described in Fig. 2. *c*, N-terminal fragment of fibrinogen (N-DSK)²⁹ was used as substrate. 20 mg of N-DSK in 2 ml of 0.2 M NH_4HCO_3 , pH 7.9, was digested (3 h, 37°C) with 0.2 BU of botroxobin. Botroxobin N-DSK, devoid of FPA, was isolated by gel filtration (Sephadex G-25) in 0.1 M NH_4HCO_3 . Botroxobin N-DSK (60 μ M) in 2 M urea containing 20 mM calcium acetate, was incubated for 60 min at 37°C with thrombin (0.8 NIH units ml^{-1}). FPA and FPB were determined as described in *a*. *d*, Dilution of the botroxobin-N-DSK-solutions was the same as described in *b*. $\times$, FPA; $\circ$, FPB.

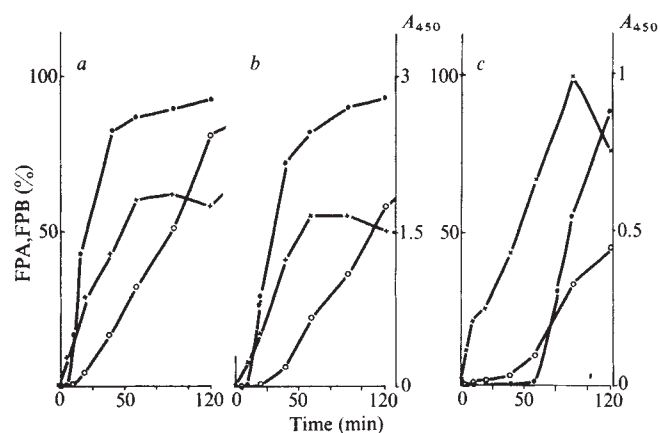


Fig. 4 Release of fibrinopeptides and polymerisation in normal and abnormal fibrinogens. Fibrinogens (fraction I-2F) from normal plasma and from two members (heterozygote and homozygote) of a family afflicted with dysfibrinogenemia Detroit were prepared as previously described, omitting the ammonium sulphate precipitation step²⁵. Stock solutions of fibrinogen (60 μ M) in 0.3 M NaCl were prepared. They were diluted with buffers at pH 7.4, containing Tris-imidazole-HCl and calcium acetate to give solutions which were 10 μ M in fibrinogen, 17 mM in Tris-imidazole and 20 mM in calcium acetate. To aliquots (200 μ l) of these solutions 1 μ l of Trasylol (1 KIE) was added. Bovine thrombin was added to a concentration of 0.025 NIH units per ml. Incubation was carried out at 37°C for various times and the digestion was interrupted by addition of hirudin (0.1 AT units). Polymerisation was determined as described in the legend to Fig. 2 except that no 'gel time' determination was made. Fibrinopeptides were determined by RIA as described in the legend to Fig. 2. *a*, Normal fibrinogen; *b*, fibrinogen Detroit, heterozygote (S.H.)^{26,27}. *c*, fibrinogen Detroit, homozygote (P.H.)^{26,27}. $\times$, FPA; $\circ$, FPB; $\bullet$, A_{450} .

fibrin formation, an activated A domain of one molecule probably interacts with an A domain of another. Evidence for another set of binding sites comes from our demonstration that the release of FPA from fibrinogen Detroit fails to produce polymerisation, although polymerisation does occur eventually after release of FPB. The A:a sites of fibrinogen Detroit are apparently not activated due to the mutation²⁵ A α 19Arg $\rightarrow$ Ser. This mutation, three amino acid residues away from the thrombin-susceptible residue (A α 16Arg) in the A α -chain, would be expected to produce conformational changes in this part of the molecule affecting subsequent interactions²⁹. We propose that the release of FPB activates a polymerisation domain (B) which interacts with another site (b) in a neighbouring molecule. Binding studies¹⁵ with thrombin-activated fibrinogen Detroit coupled to Sepharose have shown that conjugates lacking FPA as well as FPB have no affinity for normal fibrinogen. This puzzling observation could be explained if one polymerisation domain is left intact in fibrinogen Detroit, and is inactivated by the coupling of fibrinogen to Sepharose.

Our results support the suggestion²⁻⁵ that prior release of FPA is essential for the release of FPB in the presence of thrombin. Selective enzymatic removal of FPB from fibrinogen does not produce polymerisation at 37°C unless FPA is also released^{7,8}, demonstrating that the unfolding of the B:b polymerisation sites is linked with the release of FPA. It is interesting that polymer formation greatly accelerates the release of FPB. The experiments with fibrinogen Detroit strongly suggest that this effect is brought about by interactions at the B:b sites. Such interaction could induce a conformational change in a nearby B β -chain favourable to the hydrolysis of the B β 14Arg-15Gly bond by thrombin. The overall consequence of increased FPB release is an acceleration of polymer formation, an effect relevant to any discussion of thrombus formation *in vivo*.

Our results have a bearing on the role of calcium in fibrin formation. It seems that Ca^{2+} , which has only a minor effect on

the activation process itself, shortens the time for subsequent gel formation. This may account for the effect of Ca^{2+} on the opacity of clots. Complete transition from opaque to transparent clots occurs in the absence of Ca^{2+} at pH 8.5. In the presence of Ca^{2+} opaque clots are produced throughout the pH range that has been studied, suggesting that Ca^{2+} influences the ionisation of residues with $pK_s \leq 8.5$. Ca^{2+} ions possibly stabilise a conformation favourable to the formation of opaque clots, and they are known to bind to fibrinogen with high affinity at three binding sites³⁰ and to protect against thermal denaturation³¹.

Light-scattering data⁸ can be interpreted in terms of interacting domains. Those data show that the initial polymerisation geometry at pH 6–7 is predominantly 'end-to-end'⁸. At the lower end of this range, polymerisation is almost exclusively correlated to FPA release and the end-to-end geometry probably results from activation of the A:a binding sites. At pH 9–10, when lateral aggregation is a dominant feature of initial polymerisation geometry⁸, release of FPB is particularly fast and the lag phase is short. Lateral aggregation can therefore be explained by activation of the B:b binding sites after release of FPB. However, the release of FPA is also fast at high pH, indicating that the interaction at the A:a sites is hampered at high pH.

The possible mechanism of fibrin formation *in vivo*

Figure 6 represents the sequence of events in fibrin formation. If the B sites are close to FPB in the dimer of fibrinogen, an interaction involving the B site on one side of the dimer with its corresponding b site can modify the structure of the 'free' B site on the other half-molecule, so that for steric or conformational reasons the B peptide is cleaved faster by thrombin.

Our results are relevant to fibrin formation *in vivo*. The slow initial release of FPB and the presence of antithrombins in blood implies that type I fibrin is the favoured species in blood. Some indications of this were obtained in preliminary experiments with fibrin isolated from clotted whole blood. Blood was drawn into glass bottles containing inhibitors of fibrinolysis (Trasylol, 40 KIE ml^{-1} and ϵ -aminocaproic acid, 2 mg ml^{-1}). The blood

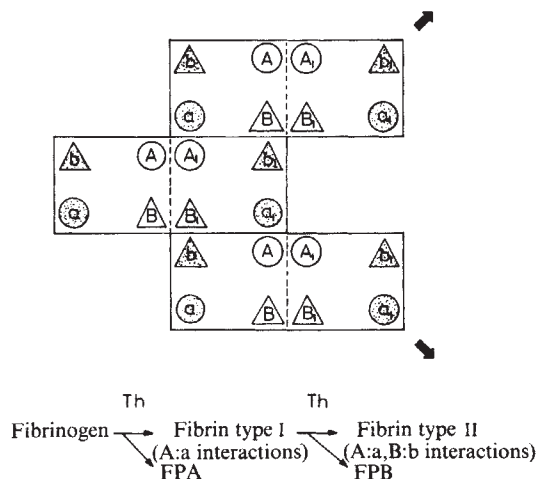


Fig. 6 Schematic representation of sequence of events in fibrin formation. The upper half of the figure shows schematically possible interactions at the sites A:a and B:b. Because of the dimeric nature of fibrinogen each of these sites is present in duplicate, that is, AA_1 and so on. The arrows show that the direction of the initial growth of the polymer is different when the A:a or the B:b sites are exclusively activated. The lower part of the picture summarises the events which occur during formation of the two types of fibrin.

was allowed to clot for 6 h at 37°C and then left overnight at +4°C. The fibrin was isolated by washing extensively with water and buffered glycine solution (pH 6.9) followed by extractions with chloroform-methanol (2:1) and ethanol (30%). The fibrin, highly cross-linked as judged by SDS-gel electrophoresis, was cleaved with CNBr and the N-DSK portion was isolated²³. SDS-gel electrophoresis showed that the purity of the N-DSK isolated from blood fibrin was the same as that of N-DSK prepared from purified fibrinogen. N-terminal analysis of N-DSK from blood fibrin showed a Gly/Tyr ratio of about 1.3:1 compared with the expected 2:1 if both FPA and FPB had been released. When N-DSK from blood fibrin was digested with thrombin (80 NIH units ml^{-1}) the change in N-terminal pattern suggested cleavage of the B β 14Arg-15Gly bond, indicating that FPB was part of the N-DSK structure in this material.

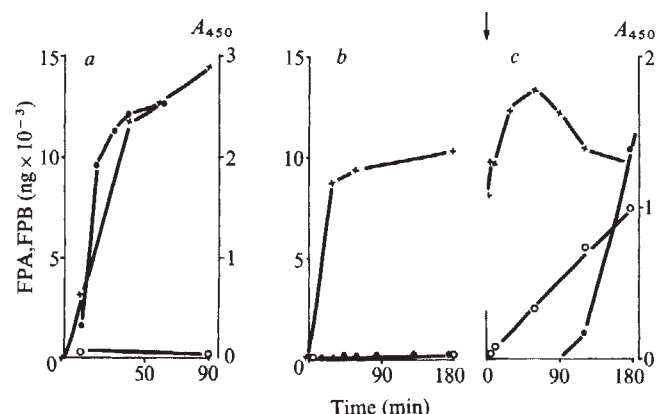
Type II fibrin may therefore form only at sites within blood vessels where the thrombin concentration is extremely high, that is, at the site of a lesion in the vessel wall where adhesion and aggregation of platelets occurs. Near the lesion, thrombin concentration rapidly decreases due to blood flow and interaction with antithrombin, and the fibrin formed is probably mainly type I. However, even a slight activation of the B site with subsequent B:b interaction may considerably change the subsequent time course of fibrin formation due to accelerated FPB release. This may result from disturbances in the composition of blood or its flow properties and may predispose it to the development of occluding thrombi in arteries and veins. It is interesting that type II fibrin seems to be more compact than type I¹⁰ and that the latter is more easily digested by plasmin³². Type I fibrin may then more easily form soluble complexes with fibrinogen which are subsequently attacked rapidly by plasmin^{33,34}.

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Fig. 5 Release of fibrinopeptides and polymerisation in normal and abnormal fibrinogen. Fibrinogens were prepared and diluted as described in the legend to Fig. 4. RIA and polymerisation curves were determined as described in the legend to Fig. 2. *a*, Normal fibrinogen. Digestion with botroxobin was carried out at a concentration of 0.02 BU per ml at 37°C. A firm clot was formed which could not be dispersed in the buffer used. Therefore, subsequent addition of thrombin gave only partial release of FPB (not shown). *b*, Fibrinogen Detroit, homozygote. Digestion with botroxobin carried out as in (*a*). *c*, Fibrinogen Detroit, homozygote, in a separate experiment was digested with botroxobin in the same conditions as in (*a*) for 100 min. At that time (arrow), thrombin was added to a concentration of 0.025 NIH units per ml and incubated at 37°C for an additional 3 h. $\times$, FPA; $\circ$, FPB; $\bullet$, A_{450} .



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Synthesis of an ovalbumin-like protein by *Escherichia coli* K12 harbouring a recombinant plasmid

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A cloned DNA transcript of ovalbumin mRNA was cut a few nucleotides away from the initiator codon, and fused in phase to the beginning of the Escherichia coli β -galactosidase gene. The hybrid gene has been cloned in E. coli where it produces large amounts of an ovalbumin-like protein.

RECOMBINANT DNA technology now permits the introduction and cloning, in *Escherichia coli* K12, of genes of various origins. However, the expression of foreign genes in this bacterial environment is still poorly documented. Several genes isolated from the lower eukaryotes (yeast, *Neurospora crassa*, *Drosophila*) have been shown to be spontaneously expressed in *E. coli*^{1–6}. There has been no report of genes cloned from the higher eukaryotes that are spontaneously transcribed or translated. Transcription can be efficiently enforced by the integration of gene sequences within a bacterial or a phage gene^{7,8}. It seems that the best way to promote translation into a polypeptide product is by the construction of hybrid genes in which the eukaryotic sequence is fused in phase to a bacterial gene. This approach has been used to construct *E. coli* strains capable of producing the small hormone, somatostatin, in the form of a hybrid protein⁹. The gene coding for the hormone, which is 14 amino acids long, was synthesised chemically, fused to the end of the *E. coli lac Z* gene coding for β -galactosidase and cloned in a multicopy plasmid. The β -galactosidase moiety (about 1,000 amino acids) was elegantly connected to the somatostatin moiety through a methionine residue. As somatostatin contains no methionine, and as the latter is labile in certain conditions, the hormone could be extracted from the hybrid polypeptide by treatment with cyanogen bromide⁹.

Most polypeptides, however, contain methionine residues, so that the above method is not of potential general use unless

other cleavable protein 'linkers' are made available. Many possible applications of genetic engineering, on the other hand, rely on the synthesis of polypeptides larger than somatostatin, coded by gene sequences too long to be chemically synthesised at present. The recent discovery that at least some eukaryotic genes are split^{10–20} makes it unlikely that such genes, isolated from the genome, are easily amenable to expression in *E. coli*. Rather, the most general approach seems to involve (1) enzymatic synthesis of the desired gene sequence from mRNA; (2) fusion of this cDNA sequence to a bacterial gene in such a way that the eukaryotic sequence can be read in the proper phase; (3) cloning in *E. coli*.

We have applied this protocol to the chicken ovalbumin gene sequence and we report here the construction of bacterial strains which synthesise large amounts of an ovalbumin-like protein. This result demonstrates the practicability of the approach and opens broader prospects to genetic engineering methodology.

The ovalbumin system

Ovalbumin is the major egg white protein. It is a polypeptide chain of 386 amino acids, with a single intra-chain disulphide bond, and undergoes a few post-translational modifications such as cleavage of the first methionine residue, *N*-acetylation, glycosylation and phosphorylation of certain residues. There are several known genetic variants^{21–26}.

Ovalbumin mRNA (ov mRNA) is predominant in the chick oviduct, which facilitates its isolation in relatively pure form. Ov mRNA is 1,859 nucleotides long. Its complete sequence has been determined by McReynolds *et al.*^{26,27}, and consists of a 64-nucleotide long 5' non-coding region, followed by a 1,158-long coding region, and a 637-long 3' non-coding region (Fig. 1).

The ovalbumin gene is split in the chicken genome^{12,16,20}. We have recently isolated, by molecular cloning, the various genomic DNA fragments which code for cytoplasmic ovalbumin mRNA^{16,17}. Analysis of the cloned fragments has revealed the presence of at least six intervening sequences, all located within translated regions of ov mRNA. As the structure of the

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Fig. 1 Map of ovalbumin mRNA. Schematic representation of ov mRNA according to McReynolds *et al.*²⁶, showing the translated (T) and non-translated (NT) regions, the AUG start signal (nucleotides 65-67) for protein synthesis, and the position of the *HhaI*, *PstI* and *SstI* sites in the corresponding DNA sequence^{26,31}.

ovalbumin gene was likely to prevent its expression in *E. coli*, we turned our attention towards the DNA transcript of ov mRNA.

Humphries *et al.*²⁸, using a protocol devised for the cloning of globin mRNA transcripts^{29,30}, first isolated a plasmid, pCRI ov2-1, which carries about 1,730 base pairs of DNA transcribed from ov mRNA. Evidence has been presented that a faithful cloned DNA copy is obtained²⁸. As ov mRNA is 1,859 nucleotides long, pCRI ov2-1 does not harbour all the ov mRNA sequence. Restriction mapping, DNA sequencing and electron microscopy analysis^{16,17,28,31}, however, indicate that the missing parts originate from untranslated regions of the mRNA.

As pCRIov2-1 carries all the sequence coding for ovalbumin, we first asked whether the gene is expressed in C600r_m⁺ (pCRIov2-1). Transcription of the ovalbumin sequence from the correct strand could be demonstrated. However, the strain produced no ovalbumin-like protein, as indicated by radioimmunoassays (see below) which were negative, even in conditions where less than one molecule per cell would have been detected (O.M.-P., unpublished). We tentatively concluded that the ovalbumin sequence in pCRIov2-1 has been accidentally fused to an unknown plasmid gene, probably out of the proper reading frame.

Construction of recombinant *lac*-ov plasmids

We then decided to fuse the ovalbumin gene sequence to the beginning of the *E. coli lac Z* gene, which codes for β -galactosidase. Fuller³² has generated a small DNA fragment of 205 base pairs, flanked by two *EcoRI* sites, which carries, in addition

to the L8UV5 *lac* operator promoter region, the information for the first eight amino acids of β -galactosidase. (The L8UV5 mutation makes the system insensitive to catabolite repression.) This fragment was engineered by Charnay *et al.*³³ into a phage, λ CI857 $\text{plac}5\Delta\text{ZL8UV5}$, the DNA of which has a single *EcoRI* site located after the eighth amino acid of β -galactosidase in the *lac* sequence. We used this DNA to generate by digestion with *EcoRI* and *HindIII* a DNA fragment of 2.85 kilobases which carries the beginning of the *lac* sequence. This fragment was recombined *in vitro* with pBR322 DNA³⁴ digested by the same two enzymes, as outlined in Fig. 2, and cloned in *E. coli*. The resulting plasmid, called pOMP0, was the vector used for further recombination experiments. pOMP0 is about 7.1 kilobases in size. It makes the recipient host cell resistant to ampicillin (but not to tetracycline) and *lac* constitutive (because *lac* repressor is overtitrated by the operator carried by the multicopy plasmid), and has a single *EcoRI* site located near the beginning of the *lac Z* gene (Fig. 2).

Most of the ovalbumin sequence carried by plasmid pCRIov2-1 can be isolated in a restriction fragment of 2,430 base pairs, *Hha*ov, obtained by digestion of plasmid DNA with *HhaI*^{12,28}. The analysis of cloned genomic parts of the ovalbumin gene¹⁷ has demonstrated the existence, near the 5' end, of a site sensitive to *HhaI*, located very close (about 30 base pairs) to an *SstI* site in a region present in pCRIov2-1 (ref. 31). When the nucleotide sequence of this region of ov mRNA established by McReynolds *et al.*²⁶ became available, it seemed that there was no *HhaI* site (GCGC) in the sequence. It occurred to us, however, that 35 nucleotides ahead of the *SstI* site closest to the 5' end (nucleotides 116-120), there was a sequence, GGUGCA (nucleotides 77-82), which could be changed to GGCGCA without modifying the amino acid sequence (GGU and GGC both code for glycine). The assumption could be made that McReynolds *et al.*²⁶ and Humphries *et al.*²⁸ had worked on different genetic variants and that the *HhaI* site which we observed would be located at nucleotides 78-81 of ov mRNA, overlapping two triplets, GGC and GCA, which code for the fifth and sixth amino acids of ovalbumin. Accordingly, the fragment extracted from pCRIov2-1 should be cut at the very beginning of the mRNA sequence coding for amino acids.

Fig. 2 Construction of plasmids pOMP0 and pOMP2. λ CI857 *plac*5 L8UV5 Δ Z was constructed by Charnay *et al.*³³ by first inserting the 205-base pair *lac* sequence (described in ref. 32) into the *EcoRI* site located at the end of the *lac Z* gene of a derivative of λ CI857 $\text{plac}5\text{L8UV5}$. Intramolecular recombination subsequently yielded a phage with only one *EcoRI* site between the end of the *lac Z* gene and the 205-base pair *lac* sequence as shown in a. 500 ng of λ CI857 $\text{plac}5\text{L8UV5}\Delta\text{Z}$ DNA (shown as a, where only the region carrying the *lac* sequence is depicted) and 50 ng of pBR322 DNA (shown as b) were digested by *EcoRI* and *HindIII*, and ligated by T4 DNA ligase in a final volume of 15 μ l as usual^{16,35}. Aliquots were used to transform CaCl₂-treated strain 478 (C600 r_m⁺recBC⁻lacy⁻) and transformants were selected on tryptone plates containing ampicillin (20 μ g ml⁻¹) and 5-bromo-4-chloro-indolylgalactoside (X-gal, 40 μ g ml⁻¹), on which strains carrying the recombinant plasmid give blue colonies. Purified pOMP0 was next used to construct recombinant plasmids harbouring the ovalbumin sequence (see legend to Fig. 3). The map of one representative plasmid, pOMP2, was established by restriction analysis using the indicated sites (see Fig. 4). pOMP0 is about 7.1 kilobases long, has single sites for *EcoRI*, *HindIII*, *SstI* and *PstI*. All experiments were carried out with ultrapure hyperhydrous water (Institut Pasteur).

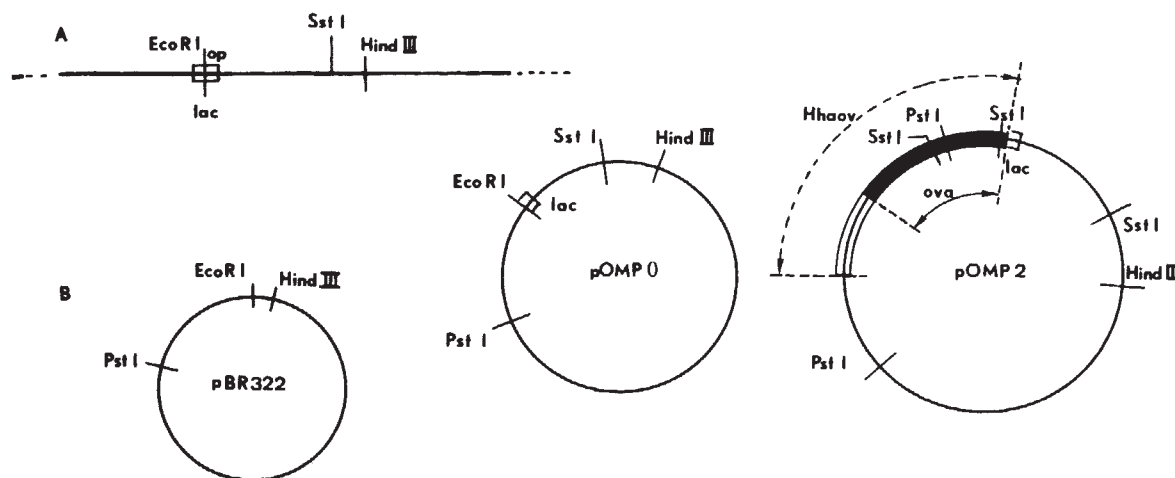


Fig. 3 Fusion of the ovalbumin sequence to the beginning of the *lac Z* gene. The DNA sequence of the *EcoRI*-restricted *lac Z* gene fragment is shown in I with the corresponding amino acid sequence³⁹. The first eight amino acids of ovalbumin and their corresponding DNA sequence²⁶ are shown in II, with one change at position 79 (C:G instead of T:A written above the line) which creates a site for *HhaI* restriction endonuclease⁴⁰. The latter splits the DNA as indicated. Elimination of the single-stranded ends and blunt-end ligation produce the fused gene sequence shown in III, with the corresponding amino acid sequence. Single-stranded ends were eliminated in the case of pOMP0 by digestion with *S*₁ nuclease followed by a treatment with DNA polymerase I (to repair possible mistakes made by *S*₁). *Hha*ov protruding ends were digested either by *S*₁ nuclease or *S*₁ nuclease followed by DNA polymerase I, or by DNA polymerase I alone making use of the 3' exonuclease

	Met	Thr ¹	Met ²	Ile ³	Thr ⁴	Asp ⁵	Ser ⁶	Leu ⁷				
I	A T G	A C C	A T G	A T T	A C G	G A T	T C A	C T G	G			β-galactosidase
	T A C	T G G	T A C	T A A	T G C	C T A	A G T	G A C	C	T T A A		
				Met	Gly ¹	Ser ²	Ile ³	Gly ⁴				
									T			
II	Ovalbumin			A T G	G G C	T C C	A T C	G G C	G			
				T A C	C C G	A G G	T A G	C C				
									Ala ⁵	Ala ⁶	Ser ⁷	Met ⁸
									C A	G C A	A G C	A T G
									G C	G T	C G T	T C G
									A			T A C
	Met	Thr	Met	Ile	Thr	Asp	Ser	Leu	Ala	Ala	Ser	Met
III	A T G	A C C	A T G	A T T	A C G	G A T	T C A	C T G	G C A	G C A	A G C	A T G
	T A C	T G G	T A C	T A A	T G C	C T A	A G T	G A C	C G T	C G T	T C G	T A C

alone making use of the S_1 exonuclease activity of this enzyme. 1 μ g of purified²⁸ pOMP0 DNA was digested by *Eco*RI (ref. 43) for 40 min at 37 °C. The reaction was stopped by heating for 10 min at 65 °C. The mixture was adjusted to 3 mM ZnSO₄, 30 mM Na acetate, 300 mM NaCl, and 20,000 units of purified *S*₁ endonuclease²⁸ were added. After 15 min at 37 °C, the reaction was stopped by heating for 10 min at 65 °C, and the DNA was dialysed against 10 mM Tris-HCl, pH 7.5, 5 mM EDTA (step I). Part of this preparation (330 ng) was treated, in the presence of all 4 dNTPs, with 0.2 units of DNA polymerase I (ref. 28) for 15 min at 15 °C and then heated for 10 min at 65 °C (step II). Part of the DNA was then treated by bacterial alkaline phosphatase (Worthington, heated for 5 min at 80 °C before use) for 15 min at 65 °C (ref. 51). The reaction was terminated by extraction with a mixture of phenol and chloroform, and the DNA was extensively dialysed against 10 mM Tris-HCl, pH 7.5, 5 mM EDTA (step III). *Hha*ov (ref. 28) fragment was treated either with: *a*, *S*₁ exonuclease (500 ng of *Hha*ov were digested by 5,000 units of *S*₁ in the conditions described for pOMP0) or, *b*, DNA polymerase I (280 ng of *Hha*ov were treated with 0.2 unit of enzyme as described for pOMP0) or, *c*, with *S*₁ exonuclease and DNA polymerase I (150 ng of the DNA preparation in *a* being treated with 0.2 unit of DNA polymerase I). 20 ng of pOMP0 DNA at steps I, II or III was ligated with 7 ng of *Hha*ov treated either as in *a*, *b* or *c* in a final volume of 10 μ l with 0.12 units of T4 DNA ligase (Biolabs) for 15 h at 12 °C (refs 9, 51). The ligated mixture was diluted to 200 μ l with 0.1 M Tris-HCl, pH 7.0, and used to transform CaCl₂-treated *E. coli* 1398 (ref. 35). Transformants were selected on tryptone plates containing ampicillin (20 μ g ml⁻¹). Colonies were directly screened by *in situ* hybridisation with ³²P-labelled *Hha*ov for the presence of ovalbumin sequence³⁵. The positive clones were tested for *lac* constitutivity on tryptone X-gal (40 μ g ml⁻¹) plates. DNA from the *lac*⁺, *ov*⁺, *ap*^R clones was then used to transform C600r_Km_K⁺ and 1442. The plasmids described here arose from the following combinations: pOMP1, 10 and 11 = step II pOMP0 + *Hha*ov *b*; pOMP2 and 7 = step II pOMP0 + *Hha*ov *a*; pOMP4 = step III pOMP0 + *Hha*ov *b*; pOMP5 and 9 = step III pOMP0 + *Hha*ov *c*.

Sequencing of pCRIov2-1 DNA³¹ later demonstrated that these assumptions were correct (Fig. 3).

The way in which the ovalbumin sequence in *Hhaov* was fused in phase to the beginning of the *Z* gene is depicted in Fig. 3. In brief, after elimination of the single-stranded ends of the *Hhaov* fragment and of *EcoRI*-treated pOMP0 DNA by treatment with S_1 nuclease and/or DNA polymerase I, blunt-end ligation with an excess of T4 DNA ligase should yield the structure shown in Fig. 3, in those strains where integration of the *Hhaov* fragment has occurred in the proper orientation. The hybrid gene is then expected to produce an ovalbumin-like protein of 389 amino acids in which the first five amino acids of ovalbumin are substituted by the first eight amino acids of β -galactosidase (including the first Met residue).

Recombinant molecules were introduced by transformation into *E. coli* 1398 (ref. 35), a lysogenic host adequate for the *in situ* colony hybridisation method which we have recently devised³⁵. Ampicillin-resistant colonies were then screened with ³²P-labelled *Hha*ov, a probe which reacts only with recombinant plasmids harbouring an ovalbumin sequence. We thus obtained eight independent strains showing *lac* constitutivity (blue colonies on X-gal plates, see legend to Fig. 3). The isolated plasmids (pOMP1, 2, 4, 5, 7, 9, 10, 11) were then transferred into a non-lysogenic host (C600r_k⁺).

Characterisation of the recombinant plasmids

The orientation of the integrated ovalbumin sequence was determined as follows: recombinant plasmids are cut by *Pst*I in two sites, one in the ampicillin gene of pBR322 (ref. 34), the other in the ovalbumin sequence¹². If the latter is properly orientated, digestion of plasmid DNA by *Pst*I should yield two

DNA fragments of about 6.9 and 2.7 kilobases. If it is not, digestion by *Pst*I should produce fragments of about 8.5 and 1.1 kilobases. The results shown in Fig. 4 indicate that the isolated plasmids do fall into these two expected classes: in plasmids pOMP1, 2 and 4, the ovalbumin sequence is in the proper orientation to be transcribed from the *lac* promoter, whereas in plasmids pOMP5, 7, 10 and 11, it is in the opposite direction.

To determine whether some of these strains produce an ovalbumin-like protein, we used a radioimmunoassay, in which iodinated ovalbumin is mixed to bacterial extracts and then allowed to react with anti-ovalbumin specific antibodies. The details of the method are described in the legend to Fig. 5. The results were perfectly correlated with the structure of the recombinant plasmids: plasmids pOMP1, pOMP2 and pOMP4 caused very strong competition, whereas the others (including pOMP0) did not. Plasmid pOMP2 was arbitrarily selected for further studies.

Production of an ovalbumin-like protein by pOMP2

Further radioimmunoassays were carried out to quantitate the amount of ovalbumin-like product made in *E. coli*. In Fig. 5, competition by pure, unlabelled ovalbumin (Fig. 5b) is compared to competition by extracts of an *E. coli* strain carrying pOMP2 (Fig. 5a). The competition by the latter is only 80% complete, which suggests that one (or several) antigenic site is missing in the protein manufactured by bacteria. Furthermore, the competition curve may be regarded as being multiphasic. This suggests either that some of the antigenic sites made in *E. coli* are more abundant than others, or that some have a lower affinity than those of native ovalbumin. Because the size of the

ovalbumin-like product is approximately that expected (Fig. 6 and below), we favour the second interpretation. As we noted above, ovalbumin, in the oviduct, undergoes some post-translational modifications which may play an important part in the structure of antigenic determinants. As it is unlikely that such modifications take place in *E. coli*, the ovalbumin-like product (which in any case differs from native ovalbumin by a few amino acids in the N-terminal end) may be, for some of the antigenic sites, a less efficient competitor than ovalbumin. On this assumption, quantitation based on the first part of the multiphasic curve indicates that 2.5×10^6 cells may produce the equivalent of about 15 ng of ovalbumin, that is, about 90,000 molecules of ovalbumin-like protein per cell. If the whole curve was considered as monophasic the estimate would be of 45,000 molecules per cell (5,000–45,000 in a variety of independent experiments, 30,000 on average).

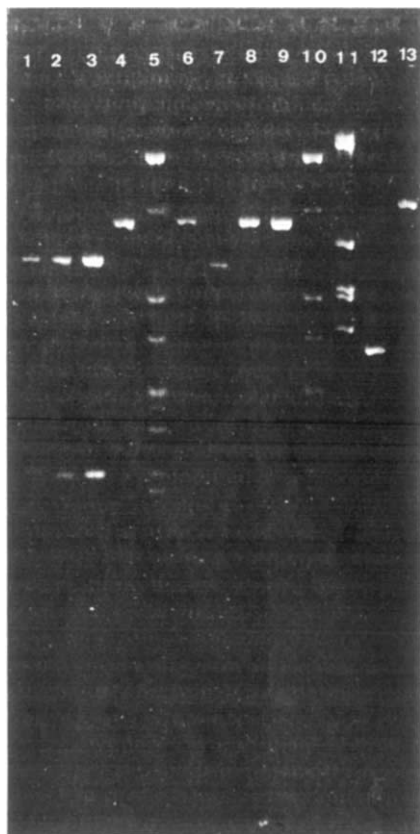


Fig. 4 Restriction analysis of plasmids carrying the ovalbumin sequence. Horizontal agarose (0.5% Sigma type II) gels (25 cm long) were run at 5 V cm^{-1} as in ref. 40. Plasmid DNAs were purified from cleared lysates by phenol extraction and ethanol precipitation, subjected to digestion by *Pst*I endonuclease (Boehringer)^{41,42}, and run in parallel with size markers (lanes 5 and 10: λ cl857Sam7 DNA digested by *Eco*RI and *Bam*HI^{43,44}, lane 11: λ cl857S7 DNA digested by *Eco*RI (ref. 43), lane 12: pBR322 DNA digested by *Hind*III (ref. 34). The size of fragments was estimated on a semilogarithmic plot relating distance to size (in kilobase pairs). Plasmids pOMP1, 2 and 4 (lanes 1, 2 and 3, respectively) show two fragments of estimated size 6.9 and 2.8 kilobases. Plasmids pOMP5, 7, 10 and 11 (lanes 4, 6, 8 and 9, respectively) show two fragments of about 8.65 and 0.7 kilobases. Plasmid pOMP9 (lane 7) does not yield the expected fragments. The total plasmid size (except for pOMP9) can thus be estimated to range over 9.35–9.8 kilobases. The theoretical size is 9.63 kilobases (using estimates of 4.35 kilobases for pBR322; 2.43 kilobases for *Hha*ov; 2.85 kilobases for the *lac Eco*RI-*Hind*III fragment (as determined by electron microscopy). The respective orientation of the *lac* and ovalbumin sequences which can be deduced from these data was confirmed, in the case of pOMP2, by a variety of other experiments including double digestion by *Pst*I and *Hind*III, which yields three fragments of about 3.45, 3.1 and 2.97 kilobases, and digestion by *Sst*I (data not shown) (see Fig. 2). Lane 13, plasmid pOMP2 digested by *Hind*III (ref. 34).

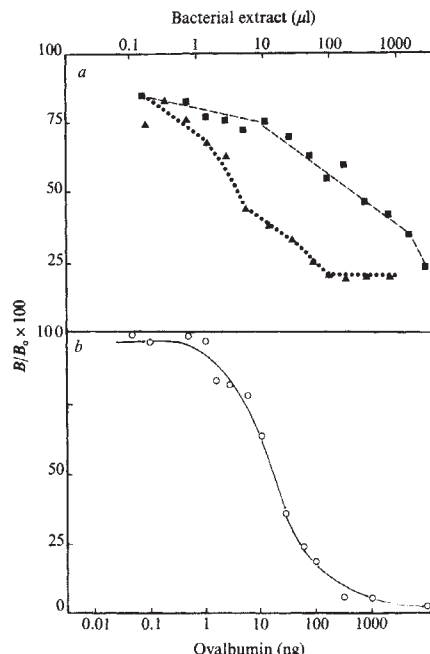
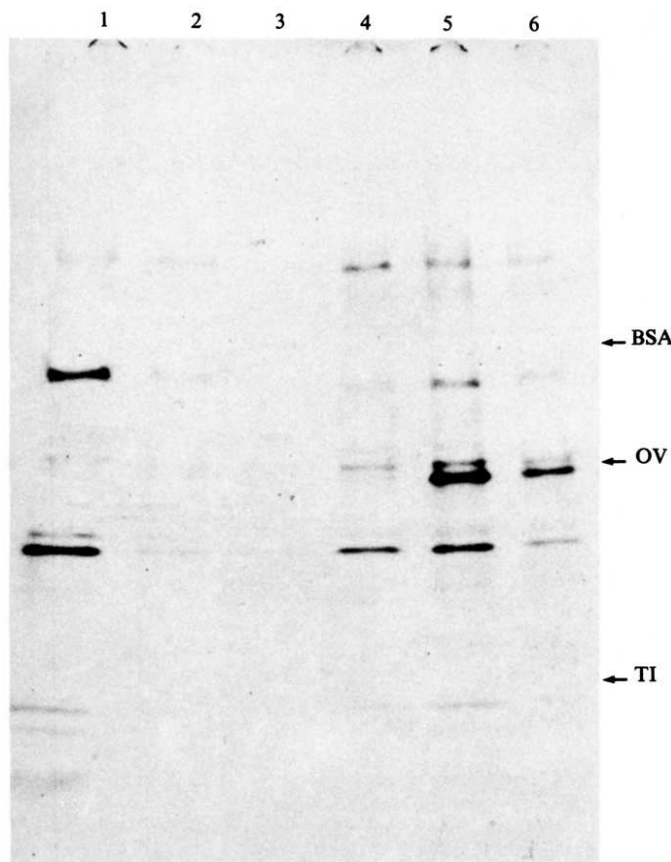


Fig. 5 Detection of ovalbumin-like protein in bacterial extracts. Ovalbumin (Sigma, 99% pure) ($30 \mu\text{g}$ in $20 \mu\text{l}$) was labelled with 1 mCi of Bolton and Hunter reagent (Amersham; $>1,375 \text{ Ci mmol}^{-1}$), according to the procedure of ref. 45, except that the reaction was stopped after 18 h at 0°C . Radioactive protein was then applied to a Sephadex-G75 column equilibrated with 0.05 M phosphate buffer, $\text{pH } 7.5$, 0.25% gelatin, and eluted with the same buffer. The iodinated product (initial specific activity $\sim 16.5 \mu\text{Ci } \mu\text{g}^{-1}$) was stored at 4°C . Antibodies against ovalbumin were raised in rabbits by injection of 1 mg of ovalbumin (purified by preparative gel electrophoresis) emulsified in Freund's complete adjuvant. A second injection was made 3 weeks later, and bleedings were made 2 weeks after. Antibodies were purified by $(\text{NH}_4)_2\text{SO}_4$ precipitation and chromatography on CM-DEAE cellulose and Biogel P11 columns⁴⁷. Radioimmunoassays were carried out in a final volume of $300 \mu\text{l}$ in PBSA (phosphate buffer 0.1 M , $\text{pH } 7.5$, $\text{NaCl } 0.15 \text{ M}$, bovine serum albumin 5 mg ml^{-1}). To the desired dilution of bacterial extract (or of unlabelled ovalbumin) in PBSA, were added $20 \mu\text{l}$ of mixture containing $15 \mu\text{l}$ of nonspecific rabbit antiserum as a carrier and $10,000 \text{ c.p.m.}$ iodinated ovalbumin. This was followed by addition of $50 \mu\text{l}$ of a dilution of the specific antibodies able to precipitate 50% of the iodinated protein. After an 18-h incubation at 4°C , antibody-bound ovalbumin was separated from free ovalbumin by addition of Na_2SO_4 18% (w/v) final. The precipitate was centrifuged for 5 min at room temperature in a microfuge; supernatants were pipetted and pellets and supernatants counted in a gamma counter. Control experiments with iodinated antibodies demonstrated that precipitation by Na_2SO_4 was essentially complete. Precipitation of iodinated ovalbumin in the absence of specific antibodies did not exceed 5–8% of input. Strain 1442 (BMH 7156 $\Delta(\text{pro}lac)$ $\text{F}'\text{lac}^{\text{O}i}\text{Z u118}$ was transformed by pOMP2, and grown on glucose minimum plates (to prevent loss of the episome ensuring the overproduction of *lac* repressor). Liquid cultures of 1442 (pOMP2) and C600 $\text{r}^{\text{O}i}\text{m}^{\text{K}}$ (pOMP2) in L broth with $5 \mu\text{g ml}^{-1}$ ampicillin were inoculated from plates at about 10^8 cells per ml and grown to about 1.2×10^9 bacteria per ml in the presence or absence of IPTG (5 or 10 mM). In some experiments cultures were directly frozen and sonicated in the presence of protease inhibitor, PTSE⁴⁶ *p*-toluyl-sulphonyl-fluoride, Ega-Chemie, $30 \mu\text{g ml}^{-1}$). In the experiment shown in *a*, bacteria were concentrated 20-fold in PBSA containing PTSE, frozen, sonicated ($2 \times 20 \text{ s}$ in the cold) and diluted serially in the same buffer mixed with a constant amount of extract of the parent strain 1441. Radioimmunoassays were carried out in duplicate. B/B_0 is the ratio between the c.p.m. precipitated in the presence and the absence of bacterial extracts or unlabelled ovalbumin (nonspecifically precipitated c.p.m. being subtracted each time). *a*, Extracts of 1442 (pOMP2) with (▲) and without IPTG (■). The abscissa has been recalculated as μl of nonconcentrated extract ($1 \mu\text{l} = 1.2 \times 10^6$ bacteria). *b*, Standard curve with ovalbumin (Sigma, 99% pure). 50% competition corresponds to 15 ng of pure ovalbumin.

Fig. 6 Size of the ovalbumin-like product in immunoprecipitated bacterial extracts. 5-ml cultures of 1442(pOMP0) (lanes 1 and 2) and 1442(pOMP2) (lanes 3–6) were inoculated at 37 °C in 63B1 glycerol medium supplemented with 5 $\mu\text{g ml}^{-1}$ ampicillin and eventually (lanes 5 and 6) with 1 mM IPTG, grown to an A_{600} of 0.5, labelled with ^{35}S -methionine (Amersham, 50 $\mu\text{Ci ml}^{-1}$, 800 Ci mMol^{-1}) for 30 min and poured over an equal volume of a crushed frozen solution containing 10 mM NaN_3 , 200 $\mu\text{g ml}^{-1}$ chloramphenicol and 30 $\mu\text{g ml}^{-1}$ PTSE. The cells were then centrifuged (5,000g, 10 min at 4 °C) and frozen at –20 °C. The pellet was resuspended on 0.5 ml of 10 mM Na phosphate buffer, pH 6.8, and sonicated (2×20 s in ice). Immunoprecipitates were prepared as described by Rhoads *et al.*⁴⁷ with minor modifications. To 10^7 trichloroacetic acid-precipitable c.p.m. were added either 5 μg of unlabelled carrier ovalbumin and 50 μg of bovine serum albumin (BSA) (lanes 3 and 5), or 50 μg of competing unlabelled ovalbumin and 5 μg of BSA (lanes 2, 4 and 6). Samples were adjusted to 1.4% Triton X-100, 1.4% sodium deoxycholate, 10 mM Na phosphate buffer, pH 7.5, and 0.15 M NaCl, to which 20 μl of a nonspecific rabbit serum was added. The mixture (210 μl) was incubated for 30 min at room temperature, layered over 200 μl of 1 M sucrose, 1% Triton X-100, 1% deoxycholate, 10 mM Na phosphate buffer, pH 7.5, and 0.15 M NaCl, and centrifuged (5 min at 4 °C in a microfuge). The upper phase was carefully pipetted and 20 μl of rabbit anti-ovalbumin antibodies, enough to precipitate 7.5 μg of ovalbumin, were added. After 60 min at room temperature, the mixture was layered over sucrose as described above, centrifuged and frozen in liquid N_2 . A 3-mm long tip was cut from the bottom of the tube and the immunoprecipitate was resuspended in 20 μl of 10% glycerol, 2% sodium dodecyl sulphate, 62.5 mM Tris-HCl, pH 6.8, 0.7 M β -mercaptoethanol and bromophenol blue, heated for 2 min at 100 °C and loaded on a sodium dodecyl sulphate polyacrylamide gel. The resolving gel was a 6–15% linear gradient of acrylamide⁴⁸. The electrophoresis was carried out with the apparatus described by Studier⁴⁹ at 4 °C for 12 h at 5 V. After the run, the gel was fixed and stained with Coomassie brilliant blue R-250 (0.2% (w/v) in methanol:water:acetic acid, 50:50:7 v/v), impregnated with diphenyloxazole, dried and fluorographed with flash-activated Fuji RX films according to the standard technique⁵⁰. Exposure was for 24 h at –70 °C. Arrows indicate the position of unlabelled size markers added to each sample: bovine serum albumin (BSA, 68,000 daltons), ovalbumin (OV) and trypsin inhibitor (TI, 21,500 daltons). The immunoprecipitates are contaminated by several bacterial proteins. This is probably due to a weak anti-*E. coli* activity of the rabbit antibodies used. Lanes 1 and 2, 1442(pOMP0); lanes 3 and 4, 1442(pOMP2) without addition of IPTG; lanes 5 and 6, 1442(pOMP2) grown in 1 mM IPTG. In lanes 2, 4 and 6, excess unlabelled ovalbumin was added as a competitor.



We could not clearly demonstrate, in C600 (pOMP2), that the synthesis of the ovalbumin-like product is under the control of the *lac* repressor (which is, at least partially, overtitrated by the operators). Because of this, we introduced pOMP2 into a strain (1442) which overproduces *lac* repressor. Synthesis was then stimulated by the *lac* inducer IPTG about 50-fold (Fig. 5a).

The size of the ovalbumin-like product was estimated as follows: extracts of cells harbouring pOMP2 and labelled *in vivo* with ^{35}S -methionine were precipitated by anti-ovalbumin antibodies, and the dissociated precipitates were loaded on polyacrylamide gels in denaturing conditions as explained in the legend to Fig. 6. The fluorograms in Fig. 6 show a predominant band corresponding to a protein which either co-migrates with or migrates slightly more rapidly than ovalbumin, depending on electrophoresis conditions. In strain 1442 (pOMP2), synthesis of this protein depends on the addition of IPTG. Addition of excess unlabelled ovalbumin in the immunoprecipitation eliminates most of the radioactive band, which indicates that it corresponds to an ovalbumin-like protein. In total labelled extracts not subjected to immunoprecipitation, the band could sometimes be directly observed. In summary, the ovalbumin-like product has a size close to that of ovalbumin.

Synthesis of foreign polypeptides by *E. coli*

We have constructed *E. coli* strains capable of synthesising an ovalbumin-like product. The available evidence indicates that: (1) synthesis of this product is under *lac* repressor control; (2) the product competes efficiently with iodinated ovalbumin in a radioimmunoassay; (3) the size of the product is very similar to that of ovalbumin.

Together, these data strongly suggest that our *E. coli* strains synthesise an ovalbumin-like product, the composition of which is probably very close to that expected, that is, eight amino acids of β -galactosidase and 381 amino acids of ovalbumin (out of the 386 of native ovalbumin). The exact structure of the hybrid protein remains to be investigated.

Bacteria harbouring the recombinant plasmids synthesise an average of 30,000 or 60,000, and up to 45,000 or 90,000 molecules per cell (depending on the interpretation of the radioimmunoassay competition curves). This corresponds to about 0.5–1% of the total protein mass of an *E. coli* cell. These figures are relatively close to the theoretical yield which can be estimated (from the number of β -galactosidase monomers synthesised in cells harbouring a multicopy plasmid with a functional *Z* gene) to be of the order of $1\text{--}2 \times 10^5$ molecules per cell.

The production of high quantities of an ovalbumin-like protein by *E. coli* strains raises several interesting points. First, the strains are perfectly stable and show no tendency to lose the recombinant plasmid. Second, ovalbumin is a secreted protein; in preliminary experiments, we find no trace of ovalbumin-like product in the supernatant of C600 (pOMP2) cultures. Third, the ovalbumin-like protein made by *E. coli* must be relatively stable in the bacterial environment, but no precise measurement has yet been made. Fourth, the codons in ovalbumin mRNA are not randomly distributed²⁶ and their frequency may differ significantly from the frequency of codons in *E. coli*. Some codons (AUC, GUG, CCA, GAC, GAA, GCA) are used more frequently in ovalbumin than in coliphage genes of known sequence^{36,37}, such as MS2 or ΦX174 . The possible deficiency of *E. coli* in the corresponding minor tRNAs does not seem to

interfere much with expression of the ovalbumin gene sequence.

This is, to our knowledge, the first example of synthesis by *E. coli* cells, of a long polypeptide chain from a higher eukaryote. The accuracy of the genetic construction which we made is not perfect, as five amino acids of the natural protein are missing and replaced by a few others from a bacterial protein. There is no theoretical reason, in our opinion, that the site of the junction between the *lac* and the ovalbumin sequences could not be moved down to the ATG initiator codon, leading to the synthesis of an accurate product. This is also the first example of a long polypeptide chain synthesised in good yield from a cloned complementary DNA made from mRNA. As a whole, our experiments demonstrate the feasibility of engineering bacteria for a potentially very large number of uses. Because of its well balanced amino acid content, ovalbumin is often used as a standard for nutritional value, and it is therefore conceivable that the ovalbumin-like product made by bacteria could be used for feeding purposes.

Biohazards associated with the experiments described in this publication have been examined previously by the French National Control Committee. The experiments were carried out accordingly in L1B1 conditions³⁸.

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Nucleotide sequence homology at 12 intron–exon junctions in the chick ovalbumin gene

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A short partial sequence homology is present at all intron–exon junctions, or splice points, in the chick ovalbumin gene; it is probably a signal for a splicing enzyme. The significance of the junction sequences for splicing is discussed. We find no evidence of strong Watson–Crick base pairing between adjacent junctions.

OUR knowledge of the organisation of the ovalbumin gene has advanced rapidly because of the successful cloning of a major part of this gene^{1–3}. Analysis of the clones¹ has shown that the gene is arranged in at least eight separate regions (I–VIII, Fig. 1), which are colinear with the mRNA sequence⁴. But these expressed pieces or exons⁵ are separated by seven sections of

DNA or introns labelled A–G which are absent in the mature mRNA. Given this remarkably complex organisation, how is the mRNA synthesised. Potentially an RNA polymerase could transcribe only the exons leaving the introns alone. This 'jumping polymerase' model⁶ would essentially join up sections I–VIII to give the correct mature mRNA. Alternatively the RNA polymerase could synthesise a copy of the entire gene copying both intron and exon regions. This pre-mRNA could be cut and the exons joined in the correct order, the introns being eliminated. The latter hypothesis seems more likely as there is evidence of a 40S pre-mRNA⁷ containing both exon and intron sequences. This mechanism also occurs for other spliced genes such as those present in adenovirus^{8–10}, the β -globin¹¹ and yeast tRNA genes^{12,13}.

The splicing must be a precise process. It has been suggested that two adjacent exons distant in an immunoglobulin λ chain

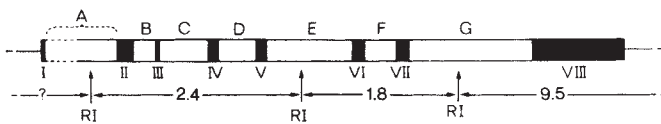


Fig. 1 Arrangement of introns (light, A-G) and exons (dark, I-VIII) in the chick ovalbumin gene. The positions of the cloned 1.8, 2.4 and 9.5 kilobase fragments, separated by *EcoRI* restriction sites are shown. The 5' *EcoRI* fragment containing exon I has not yet been characterised so that the length A is unknown.

pre-mRNA sequence⁶ could be brought into close proximity by the formation of a hydrogen-bonded duplex region between the two ends of the respective junction regions. The ligating activity of the splicing enzyme or enzymes could then join up the two exon regions in the pre-mRNA correctly. Here we present the sequence of intron-exon junction regions of the ovalbumin gene. By sequencing many such junctions we hoped to be able to decide whether a similar model might be operating, or whether other primary sequence features might also be important.

Homology at the intron-exon junctions

The major part of the ovalbumin gene is present in the 1.8 and the 2.4 kilobase fragments derived by *EcoRI* restriction of the gene and these two fragments contain 12 of the 14 known intron-exon junctions (Fig. 1). Clones of these fragments contained within the plasmid pBr 322 and the vector *Escherichia coli* χ 1776, had been previously isolated and mapped to locate the major restriction enzyme sites^{1,3}. Suitable restriction fragments were then prepared labelled at their 5' end with ³²P so that all these junction regions could be sequenced by the method of Maxam and Gilbert¹⁴. Figure 2 shows two examples of the gel patterns used to derive the sequence of junctions VI/F and F/VII in the 1.8-kilobase fragment. These and the other 10 ovalbumin junction sequences are collated in Fig. 3 together with three additional junctions sequenced in a mouse immunoglobulin λ chain⁶ and a mouse β -globin¹¹ gene. The underlined sequences are exons, defined by their presence in the mRNA. In all cases there is a terminally redundant sequence at adjacent junctions (boxed in Fig. 3) although the redundant length varies from one to four nucleotides. The effect of this redundancy is to obscure the exact point of cutting and joining of the pre-mRNA so that in no case can we deduce the precise internucleotide bond involved in splicing. For example, for junctions II/B and B/III (Fig. 3) the mRNA sequence may be derived by cutting at junction II/B at any one of the five bonds indicated by arrows in the sequence A⁺A⁺G⁺G⁺T⁺T. [This is presented here as a DNA sequence, but of course exists with U (uracil) replacing T (thymine) in the pre-mRNA. The term exon is also used interchangeably in either DNA, pre-mRNA or mRNA.] Exon II is then joined to the corresponding point cut within the same sequence in junction B/III so as to regenerate this same sequence in the mRNA.

Table 1 A consensus sequence at the intron-exon junctions

Nucleotide	Frequency at each position*							
	a	b	c	d	e	f	g	h
C	4	1	11	1	0	1	0	2
A	3	4	3	12	0	0	5	8
G	0	3	1	0	13	13	2	2
T	8	7	0	2	2	1	8	3
Consensus sequence	N	N	C	A	G	G	N	N
Less specific	T	T	C	A	G	G	T	N

* Positions a-h cross-reference to the positions marked in Fig. 3.

The redundant sequences all vary, yet are related in that they are all contained within the hexanucleotide (5') T-C-A-G-G-T (3'). They undoubtedly reflect a basic sequence homology at all junctions. A 'consensus' sequence C-A-G-G may be derived (Table 1) by counting the frequency of each nucleotide at positions a-h of Fig. 3. The base at each position is present in at least 11 of the 15 examples. But C-A-G-G is an average and individual splice points vary considerably. Specifically (see Fig. 3) seven junctions contain the consensus C-A-G-G, five contain

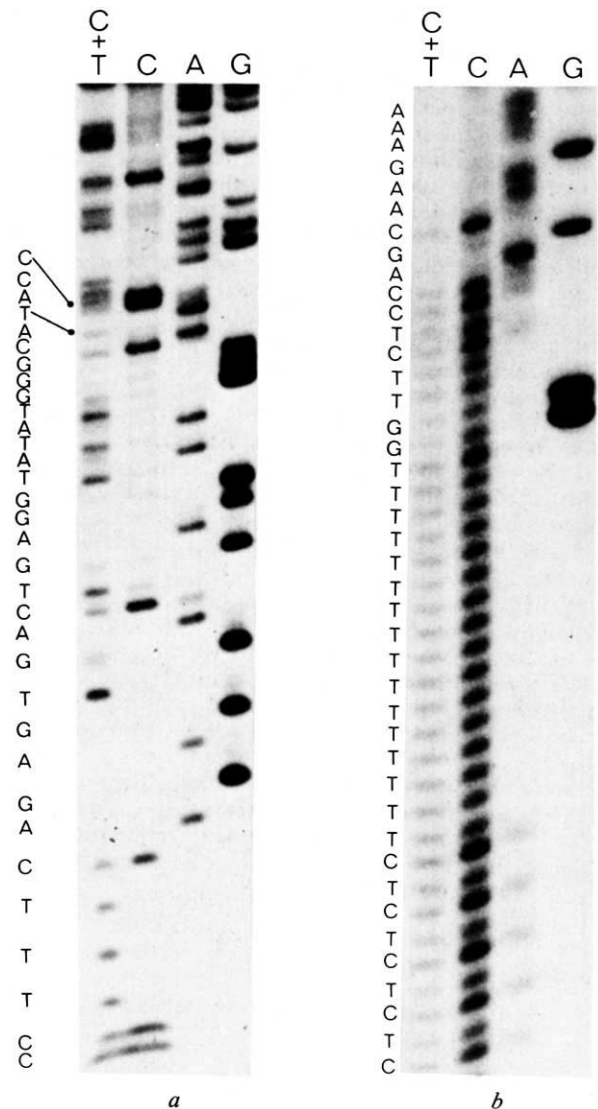


Fig. 2 a, Sequence of junction VI/F (see Fig. 3) derived from a 75 base-pair fragment of an *MboII* digest of the plasmid containing the 1.8 kilobase *EcoRI* fragment of the ovalbumin gene. *MboII* sites were labelled at their 5' phosphate end group with ³²P by the *PstI* labelling procedure described previously⁴. Complementary strands were separated by heat denaturation in formamide and electrophoresis on a 20% acrylamide, 7M urea gel (G.G.B. unpublished). Separated strands, one of which is degraded here, were subjected to chemical modification according to Maxam and Gilbert¹⁴ using the alternate G and A degradations and fractionated on a 20% acrylamide, 7M urea thin gel (0.3 mm × 20 cm × 40 cm, Sanger and Coulson²⁴). b, Sequence of junction F/VII (see Fig. 3) derived from a *HinfI*-labelled fragment of the same plasmid (a) recut with *HaeIII*. The ³²P end-labelling method was identical to that described in (a) except that the venom phosphodiesterase digestion was omitted. Final fractionation was accomplished by electrophoresis on an 8% acrylamide 7M urea long thin gel (0.3 mm × 20 cm × 80 cm) at 2.4 kV for 8 h to increase resolution at long distances (100–150 base pairs) from the 5' terminal label. The 'C' channel was severely contaminated with T residues in this experiment due to the use of 'old' hydrazine. Nevertheless the sequence was clear because of weak C bands in the 'A' channel.

three of the four bases and three (A/II, IV/D and V/E) have only two bases in common. If the averaging is extended, a less specific sequence T-T-C-A-G-G-T emerges (Table 1). Further analysis of the nucleotide frequencies on the 5' side shows that T residues are preferred and this is particularly noticeable at the 3' end of introns. An impressive example is junction F/VII (Figs 2b and 3) where a 'run' of 16 T residues occurs. Thus T residues are common in the intron sequence immediately preceding (5' of) a junction.

Splice points in the mRNA

Figure 4 shows the accurate location of the seven splice points superimposed on the ovalbumin mRNA sequence⁴. This confirms the previous conclusion that the gene is split into eight pieces¹ and that the splice points are all located in the 5' half of the mRNA. The 'limits' of the individual splice points are indicated, the uncertainty arising from the terminal redundancy at the ends of each intron (see above). For splice points (1) and (7) we do not know whether a similar redundancy exists as the complete intron sequence was not included within the 2.4 and 1.8 kilobase DNA fragments studied here.

It would seem likely, however, that splicing should occur at a unique position within the homologous sequence. We can speculate that it occurs at the arrowed position:



as only this position is common to all the redundant sequences (Fig. 3). Kourilsky and Chambon²⁵ have extended this argument and we can formulate the following simple rule. The first cut by the splicing enzyme occurs immediately 5' to a G-T doublet in an intron, and the second cut immediately 3' to an A-G doublet at the other end of the same intron. This rule works without exception for all the known ovalbumin splice points as well as for the immunoglobulin light chain (see Fig. 3).

The separate exon regions vary from the smallest 49±4 residues in length between splice points (2) and (3), to the longest extending 1,030 residues from splice point (7) to the end of the mRNA. Splice (1) occurs in the 5' non-coding region of the mRNA clearly indicating that residues 1–45 of the mRNA have some important function to perform. Possibly they are needed because they form a highly stable hairpin loop near the cap⁴ which is needed for efficient initiation of translation. An analogous splicing of a 'leader' sequence occurs in adenovirus^{8–10} and SV40 DNA^{15,16}.

Significance of the 'consensus' sequence C-A-G-G

Part of the explanation of the consensus sequence is probably the A-G and G-T doublets defining the splice point (see above). However these doublets alone would not give the observed extensive homology. Presumably this homology is part of the recognition sequence for a splicing enzyme but it is not sufficiently conserved, nor long enough to be a unique signal for a splicing enzyme or enzymes. For example, in the 50% (3,400 residues) of the entire ovalbumin gene that we have sequenced (G.G.B. unpublished results), we find 153 positions of which a 3 out of 4 match for this consensus sequence occurs¹⁶. C-A-G-G occurs at 13 of these points and only three of these positions are splice points. Nevertheless the consensus sequence is well conserved among the 12 splice points in the chick ovalbumin gene when compared with the other genes (immunoglobulin, λ and β -globin) in mice, providing strong evidence that it is conserved in evolution. But it does not occur in the spliced tRNA genes in yeast although they may have their own special mechanism as the introns are very short. The basic homology at all the splice points (excepting the tRNA genes) suggests that we are dealing with a single splicing enzyme or at least a small number of closely related enzymes, with a wide specificity amongst different genes. The alternative view that the homology results from the convergent evolution of many different splicing enzymes—for example, one for each splice point—seems unlikely as there is no logical reason why a common sequence

ovalbumin gene		a b c d e f g h																																
A/II	1	-	C	T	G	T	T	C	G	T	T	G	C	T	C	A	C	A	A	C	T	C	A	G	A	C	T	T	C	A	C	A	T	
II/B	2	}	C	A	G	C	A	C	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	
B/III			A	C	T	A	T	T	T	T	T	C	A	A	T	T	A	C	A	C	T	T	G	A	A	A	A	A	A	A	A	A	A	A
III/C	3	}	G	A	G	A	C	A	T	T	G	A	A	G	C	T	C	A	G	A	A	A	A	A	A	A	A	A	A	A	A	A	A	
C/IV			T	T	C	T	T	C	T	T	T	G	T	A	T	C	A	G	T	G	C	C	A	C	A	T	C	T	T	C	A	A	A	A
IV/D	4	}	A	C	A	G	A	T	A	C	C	A	A	T	C	T	C	C	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	
D/V			T	C	A	A	T	C	T	T	C	T	T	T	A	C	A	G	T	T	C	A	A	A	A	A	A	A	A	A	A	A	A	A
V/E	5	}	G	G	T	A	C	A	A	G	T	C	A	C	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	
E/VI			T	T	C	T	A	T	T	C	A	T	T	T	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
VI/F	6	}	T	G	C	T	T	T	C	A	G	A	G	A	G	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	
F/VII			T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	
VII/G	7	-	A	A	G	T	C	T	C	A	G	G	C	T	T	G	A	C	A	G	A	T	G	G	C	C	T	A	G	A	A	G	T	G
Others																																		
lgG(X)	T C T C C T G G C C T C T C T G C T C A G C T C A C C A C C C T T T C T A C A C T																																	
lgG(X)	G A T T T C T T A C C T G T T T C A G C A G C C A G T T C C C A G G C T G T T																																	
-globin	G T G C A T C C T G A C A A C T T C A G G C T C A G T T G A T G C G C A C C																																	

Fig. 3 Sequence at the 12 intron-exon junctions in the ovalbumin gene compared with three other junctions in other genes. For the nomenclature of the ovalbumin junctions see Figs 1 and 4. The terminally redundant sequences (boxed) at each end of an intron were vertically aligned to generate the correct mRNA sequence (Fig. 4) and to maximise homology. Exons are underlined.

should arise. We would expect in that case many different, but unique sequences as occurs for example in the recognition sequence of the many different restriction enzymes¹⁸. We cannot use this homology to definitively predict splice points as the consensus sequence is not characteristic enough. Presumably the additional features, such as the high T content at the 3' end of introns (although a computer analysis shows that this property is not restricted to these regions) and other unknown features allow specific recognition of authentic junctions whilst avoiding other regions.

No obvious base-pairing between adjacent intron-exon junctions

The splicing enzyme needs to do more than recognise specific junction regions. We presume that it must cut and join the correct number of exons in the correct order. Where there are so many (eight) pieces to be joined in a specific order, it would seem extremely wasteful to allow ligation to occur by passive alignment of the correct ends by random diffusion (analogous to the blunt-ended ligation of DNA by the bacteriophage T4 ligase). We favour the hypothesis that a specific mechanism exists to bring the distant exon sequences into close contact. The most obvious hypothesis⁶ is that base-pairing occurs between adjacent junctions, thus generating a large hairpin loop out of the intron. A computer search was made for all secondary structures¹⁹ in Fig. 3 (allowing G-C, A-T and G-T base pairs) that would approximate adjacent junctions. But the free energies (ΔG) of the best structures obtained were never less than a few kilocalories²⁰. In every case a more favourable base pairing was possible with known sequences (G.G.B. unpublished) elsewhere in the gene. Despite our inability here to find any positive evidence for a secondary structure model relying on base pairing, we cannot eliminate this mechanism. It is possible that intrinsically quite unstable structures in the pre-mRNA are stabilised by other (tertiary) interactions. For example, there may be long range interactions with other regions in the pre-mRNA or even with other RNA molecules⁶. A more definite analysis of this may be possible when the entire structure of the ovalbumin gene is known. We have also considered models based on the specific affinity of the splicing enzyme(s), or subunits of this, for one another. But such models become attractive only if there are specific and different enzymes for each splice point, as only then could correct splicing be ensured.

Conclusions

The sequence analysis of the 12 intron-exon junctions in the chick ovalbumin gene shows quite clearly that a consensus sequence is present at the splice point. But this homology is only

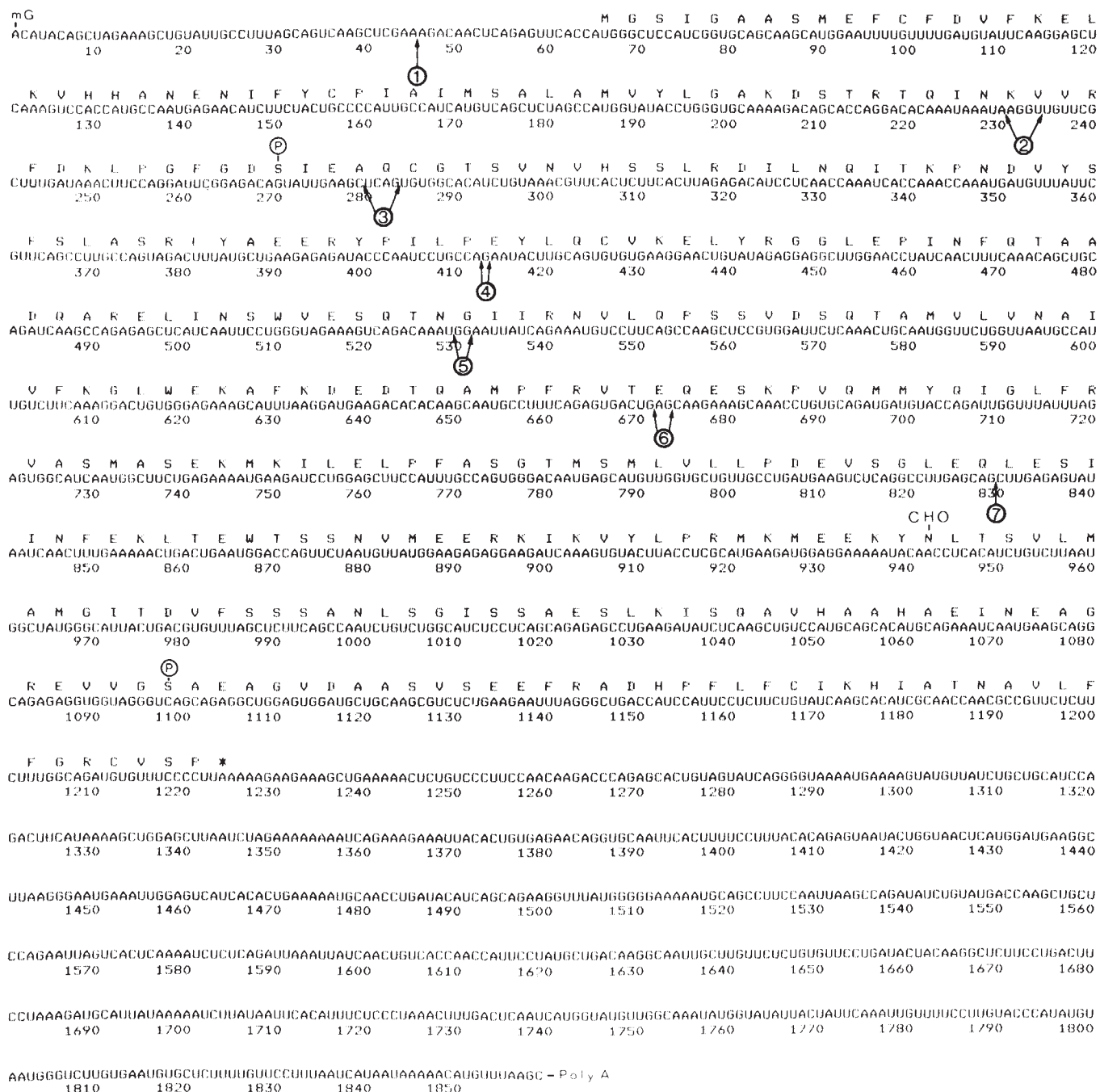


Fig. 4 The seven splice points in ovalbumin mRNA indicated by symbols ①–⑦. In no case is the splice point precisely known because of terminal redundancy at the ends of the respective intron regions.

partial. This is reminiscent of sequence studies of other important controlling regions such as the promoters in DNAs²¹ or ribosome binding sites in messenger RNA²². Unfortunately the sequence analysis has not elucidated the mechanism by which adjacent exon regions are brought into close contact for splicing. It does seem unlikely that strong Watson-Crick base pairing between adjacent junctions occurs, although we cannot eliminate weak interactions stabilised by other factors. Clearly a direct study of the enzyme or enzymes involved is needed for a more complete understanding of this problem²³.

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letters to nature

A larger and more turbulent Universe

THE modern version of the Hubble diagram in which the log of the redshift is plotted against apparent magnitude corrected for redshift and absorption effects of clusters of galaxies, is in principle capable of yielding several cosmologically useful pieces of information. In this letter I report evidence for a significant and substantial departure from the expected redshift for the Virgo cluster of galaxies. A peculiar velocity of $658 \pm 96 \text{ km s}^{-1}$ is derived but because of Virgo's membership in the Local Supercluster, such a value is not unexpected. Thus the turbulence recently observed by Smoot *et al.*¹ for the Galaxy's motion within the Hubble expansion is present elsewhere in the Universe as well. Because the Virgo cluster does not exactly follow the Hubble law, a new value for the Hubble constant equal to $43.4 \pm 2.6 \text{ km s}^{-1} \text{ Mpc}^{-1}$ is found leading to an observable Universe $1/4$ to $1/3$ again as large as was previously recognised.

When objects with reasonably consistent and sufficiently large luminosities, such as members of clusters of galaxies, are plotted in the Hubble diagram, the mean relation will have the theoretical slope of $1/5$ and a dispersion, which because of the nature of a log plot, is greater at low redshifts. The redshift residual of a cluster, if sufficiently greater than the standard deviation about the mean relation, gives the cluster's peculiar radial velocity. If the absolute magnitude of a cluster object is known, its apparent magnitude and the redshift taken from the mean cluster relationship yield the Hubble constant, H_0 . In addition, the curvature of the mean relationship at very large redshifts can give information on galaxy luminosity evolution and the deceleration parameter.

Table 1 Data for clusters of galaxies

Cluster	$c\Delta\lambda/\lambda_0$ (km s^{-1})	Δv (km s^{-1})	$\log cz$	V^*	K_V	A_V
Virgo	$1,144 \pm 68^{6,7}$	361 ± 55	3.1782	10.0^{16}	0.01	0.01
A1367	$6,424 \pm 238^8$	371 ± 57	3.8328	14.32^4	0.04	0.01
A1656	$6,943 \pm 61^9$	315 ± 48	3.8614	14.7^{17}	0.05	0.00
A2199	$9,002 \pm 180^{10-13}$	66 ± 10	3.9577	15.4^{17}	0.06	0.09
A151	$15,749 \pm 309^6$	-328 ± 50	4.1876	16.4^{17}	0.08	0.00
A754	$16,256 \pm 205$	331 ± 51	4.2202	16.78^4	0.08	0.26
A2065	$21,384$ $\pm 298^{6,14*}$	161 ± 25	4.3336	$17.10^{4,17}$	0.10	0.04
A2670	$22,491 \pm 300^{15}$	-379 ± 58	4.3441	17.3^{18}	0.11	0.01

The first column gives the cluster identity, the second the redshift in km s^{-1} referred to the Sun, the third the velocity correction of Smoot *et al.* in km s^{-1} , the fourth the log of the relativistically combined redshifts, the fifth the inflection point visual magnitude, V^* , the sixth the K -correction from Schild and Oke⁵, and the seventh the galactic absorption computed from $A_V = 0.19 (\csc |b^{\text{II}}| - 1)$. The redshift of cluster A754 is an average of three unpublished redshifts which I obtained with the image tube spectrograph and 2.1-m telescope of Kitt Peak National Observatory in February 1975 at a reciprocal dispersion of 111 Å mm^{-1} . The data represent the combined results of several thousand galaxy magnitude measurements and several hundred galaxy redshift measurements.

* Because of a calibration error in ref. 14, all redshifts were decreased by 121 km s^{-1} .

Although we are far from learning a great deal about evolution and the deceleration parameter from the Hubble diagram, it is now possible to say something about H_0 and the peculiar velocity of at least one of the nearer clusters. This has come about through the use of a reasonably accurate way to gauge relative cluster distances and through a better knowledge of the cluster redshifts. Whereas use of the brightness of the first-ranked cluster galaxy as a distance indicator yields a standard deviation of 0.30 magnitudes about the mean Hubble relation², use of the inflection point in the cluster luminosity function yields a standard deviation almost 3 times smaller^{3,4}. Following the detection of an anisotropy in the cosmic blackbody radiation¹, explained as solar motion of $390 \pm 60 \text{ km s}^{-1}$ relative to the average Hubble expansion in the direction of right ascension and declination, $\alpha = 11\text{h}$, $\delta = 6^\circ$ (or galactic longitude and latitude, $l^{\text{II}} = 248^\circ$, $b^{\text{II}} = 56^\circ$), it becomes possible to correct the observed cluster redshifts. For distant clusters this correction is fractionally small but for the nearest ones, proportionally larger changes occur. For the Virgo cluster in particular, the derived peculiar velocity is found to be significantly larger.

Table 1 presents the data for eight clusters. The straight line in Fig. 1 is the best fit with the theoretical slope $1/5$ to the 7 most distant clusters and has an intercept on the $\log cz$ axis of 0.9319 ± 0.0091 . Such a fit must be made only to data points with redshifts large enough for the effects of any peculiar velocities to be proportionally small. Because Virgo's corrected redshift is only $1,507 \text{ km s}^{-1}$ and its derived peculiar velocity is almost half this value, the Virgo point must be excluded from the fit. For the most distant cluster (A2670), a peculiar velocity equal to that of Virgo's would be only 3% of the corrected redshift and would result in an additional residual about the mean line of only 0.0137 in $\log cz$ or 0.07 mag.

The standard deviation for the fit of the seven most distant clusters about the straight line is only 0.12 mag. This dispersion is by far the smallest of any of the methods of gauging relative cluster distances. The cosmic dispersion of M_V^* must be even smaller than this value because the precision in determining V^* from the luminosity functions is at present about ± 0.05 mag, the galactic absorption is most likely 'spotty', the cluster redshifts have a non-zero mean error, and the clusters other than Virgo may have peculiar radial velocities as well. A trial fit excluding also the second nearest cluster (A1367) reduces the standard deviation to only 0.07 mag.

The Virgo cluster point lies 1.25 mag to the left of the mean relation for the seven clusters but because the residual is greater than 10σ , the Virgo cluster is interpreted as having a peculiar radial velocity of 658 km s^{-1} . The various sources of uncertainty give the standard deviation of this value as 96 km s^{-1} ; thus the Virgo peculiar velocity is certain at almost the 7σ level. An earlier preliminary calculation without the velocity correction gave a peculiar velocity about one half as large¹⁹. This result contradicts the assertions of Sandage and Tammann²⁰ who discount any peculiar velocity for Virgo and argue that the local velocity field is as quiet as it can be²¹.

The Virgo peculiar velocity indicates velocity irregularities on a scale size of about 20 Mpc. Such a condition is not unexpected because the Virgo cluster is a member of the Local Supercluster and must move within it. Following Abell¹⁷, for a supercluster of this size in equilibrium, with a mass of 10^{16} solar masses, and an isotropic velocity field, a radial velocity dispersion of about

850 km s⁻¹ is predicted. The peculiar velocities exhibited by the Galaxy (including its neighbours in the Local Group) and the Virgo cluster may be examples of the motions of clusters within bound superclusters and therefore manifestations of a universal turbulence existing since the early stages of the Universe.

If on the other hand the Local Supercluster is not bound, a cluster with a large peculiar velocity will eventually catch up to clusters with the average Hubble expansion and thus the peculiar velocity will die out with time. In the past the peculiar velocities of clusters would have been even larger. A third possibility, suggested by Rowan-Robinson²², is that entire superclusters are in rapid motion relative to each other. As superclusters are not known to be bound to each other, this motion would also die out with time and would also have been greater in the past. This third situation is not inconsistent with either of the first two—although the first and second are incompatible with each other—and we may in fact be experiencing some combination of motions.

An apparent inconsistency between the solar motion of Smoot *et al.*¹ and that of Rubin *et al.*^{23,24} deserves comment here. From a study of 96 galaxies with $3,500 < cz < 6,500$ km s⁻¹ Rubin *et al.* derive a solar motion of 600 ± 125 km s⁻¹, but in a direction about 109° away from that of Smoot *et al.* However if Rubin *et al.*'s sample of galaxies has a peculiar velocity of 815 km s⁻¹ relative to the average Hubble flow and in a direction toward $\alpha = 12.5$ h, $\delta = 33^\circ$ ($l^\text{II} = 298^\circ$, $b^\text{II} = 30^\circ$) the discrepancy is explained. The picture that is emerging at present therefore is of a Universe that is much more turbulent than previously thought. The quiet velocity field of Sandage and Tammann then is explained by the absence of a correction for the Galaxy's peculiar motion and a less accurate distance indicator (their results yield 34 km s⁻¹ for the Virgo peculiar velocity).

The Hubble constant is derived from the mean cluster predicted redshift of 847 ± 38 km s⁻¹ for Virgo and its distance in Mpc's. Table 2 lists several distances for Virgo taken from the literature. Because the Virgo peculiar velocity was not previously known, the Hubble constants derived here are

Fig. 1 The Hubble diagram for cluster of galaxies based on the inflection point magnitude, V^* , as a distance indicator. For the best fit to the seven most distant clusters with the theoretical slope of 1/5, the standard deviation is only 0.12 mag. The error bars given the mean errors of the corrected redshifts. Except for the two nearest clusters the mean errors are too small to be shown. The large residual for the Virgo cluster point at the lower left indicates a substantial peculiar velocity for this cluster.

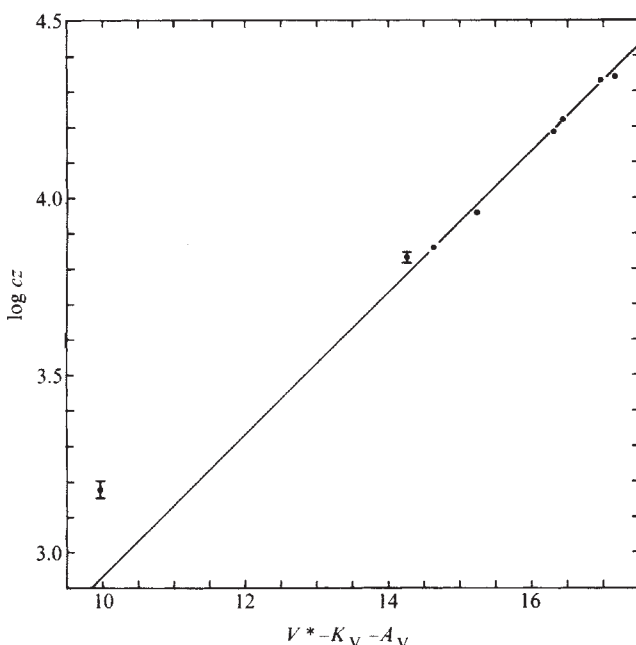


Table 2 Distance of the Virgo cluster and the Hubble constant

Reference for distance	Corrected $(m-M)_{AB}$	$(m-M)_T$	$D(\text{Mpc})$	H_0 (km s ⁻¹ Mpc ⁻¹)
25, 26	30.9 ± 0.2	30.62 ± 0.2	13.3 ± 1.2	64 ± 7
27, 26	31.3 ± 0.5	31.02 ± 0.5	16.0 ± 3.7	53 ± 13
28, 26	31.9 ± 0.4	31.62 ± 0.4	21.1 ± 3.9	40 ± 8
20	—	31.45 ± 0.09	19.5 ± 0.8	43.4 ± 2.6

Column one gives the reference, two the apparent blue distance modulus corrected as described below, three the true distance modulus, four the distance in Mpc's, and five the Hubble constant in km s⁻¹ Mpc⁻¹. Racine's²⁵ apparent blue modulus has been increased by 0.2 mag to account for a recalibration of his photographic B magnitudes given by Ables *et al.*²⁶. Racine's distance is based on a comparison of globular cluster brightnesses in M87 with those in M31 and the Galaxy. Because the Galaxy is deficient in globular clusters this distance may be too small. Sandage²⁷ and van den Bergh²⁸ compared globular cluster brightnesses in M87 with those in M31 alone. Since their results are based on Racine's measurements however, their moduli have been increased by 0.2 mag also. For these three values the true distance modulus was obtained by subtracting 0.28 mag to account for galactic absorption and K -dimming (0.26 and 0.02 mag, respectively). Sandage and Tammann²⁰ give only a true distance modulus based on the brightnesses of Virgo spirals, with the spiral calibration being based on the diameters of HII regions.

significantly smaller than those derived previously. Racine had reported $H_0 = 79 \pm 12$, Sandage 75.3 ± 17 , van den Bergh 60 ± 12 , and Sandage and Tammann a local value of 57 ± 3 and a global value of 55 ± 6 km s⁻¹ Mpc⁻¹. The new values of the Hubble constant in Table 2 are smaller by 19–33%.

On the basis of the least formal error in the distance modulus, the best estimate for the Hubble constant is $H_0 = 43.4 \pm 2.6$ km s⁻¹ Mpc⁻¹ which leads to an observable Universe about 1/4 to 1/3 larger than was previously recognised. The corresponding Hubble time (time since the beginning of the Universe if there was no slowing of the expansion) is $22.5 \pm 1.4 \times 10^9$ yr. As the oldest known stars are about 15×10^9 yr old, there may have been some slowing of the expansion and/or a delay of star formation, possibly due to the turbulence.

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Relativistic jets and beams in radio galaxies

RADIO-ASTRONOMICAL observations have recently clarified the link between the components of extended double sources and the primary power supply in the central galactic nucleus. The new data vindicate the general idea¹⁻⁴ that power is continuously supplied by beams; it seems, furthermore, that the beams are collimated in a scale little larger than the central power supply ($\ll 1$ pc), and that the orientation remains fairly steady over the whole lifetime. The giant double source 3C236, 2×10^7 light yr in total extent^{5,6}, has a compact central component aligned with the overall axis⁷; a similar phenomenon is observed in Cygnus A (ref. 8). In NGC6251, a straight jet 200 kpc long⁹ emanates from a 'blowtorch' ≈ 0.1 pc wide in the galactic nucleus¹⁰. There is a radio jet¹¹ in 3C147 reminiscent of the well-known features in M87 and 3C273; and very long baseline interferometry (VLBI) reveals linear structure in several compact extragalactic sources¹². It is argued here that collimation occurs close to a central collapsed object, and that the beams are orientated along its spin axis. Strong-field gravitational effects then stabilise the beams against jitter even if the gas fuelling the source has an inconstant flow pattern. Radio galaxies where the beam axis seems to have gradually drifted or swung, rather than pointing in a constant direction, may belong to a special class that have experienced collisions and recurrent nuclear activity.

The beams squirt from active nuclei with velocities approaching c . The most direct indications of relativistic bulk motions are the 'superluminal' variations¹² in compact sources: the detailed kinematics are still obscure—from a surfeit¹³, not an absence, of acceptable models—but most ideas entail bulk plasma motions at speeds $\sim c$. Indirect evidence for collimated relativistic outflow comes from the polarised optical continuum outbursts in objects such as A0 0235+164 (ref. 14), which pose severe interpretative problems unless the emitting material is directed towards us at speed $\sim c$ (ref. 15). Doppler-favouritism of the approaching beam, when speeds are relativistic, yields a natural explanation for the onesidedness of jets in, for instance, M87 (refs 16–18).

The central 'engine'—already inferred from source energetics to be highly efficient—seems therefore able to generate plasma whose mean kinetic energy per particle approaches the rest mass energy. Prime candidates for efficient engines are black holes of $\sim 10^8 M_\odot$ accreting from their surroundings: efficiencies can then attain $\sim 10\%$, and the output can persist for the whole $\sim 10^8$ yr lifetime of a giant source like 3C236.

Many variants of this scheme have been theoretically investigated, and almost all can generate the required high mean energy per particle^{19–27}. This is basically because the energy release is concentrated within a few Schwarzschild radii ($r_s \approx 3 \times 10^{13} (M/10^8 M_\odot) \text{ cm}$) where the keplerian speeds are $\sim c/2$, as is the sound (or Alfvén) speed in pressure-supported gas. Possible processes near the hole may involve high ion temperatures^{22,27}, relativistic shocks, radiation pressure, magnetic flares^{23,24}, or pulsar-type electromagnetic processes^{25–27}; but all options suggest that a relativistic electron-ion (or e^+e^-) plasma may be the primary product of the gravitational energy released by captured material.

Collimation of this relativistic plasma must demand some asymmetry in the surroundings, probably due to rotation. For instance, if the central power-source is embedded in a rotationally flattened cloud, the plasma will tunnel its way out by excavating nozzles along the rotation axis. This 'twin exhaust' configuration³ (which can create directed beams even if the plasma is generated with an isotropic pressure) could be established on any scale: in particular, there is no reason that the nozzles should not be at $\leq 100 r_s$ if the outflowing plasma originates very near the hole.

The nozzle walls could be confined by hot inwards-spiralling material forming a thick disk or 'donut', centrifugally inhibited from draining into the hole^{29–32}. This confining material would be supported by the pressure of radiation, ions, or magnetic fields. The shape of the nozzles depends on the precise pressure distribution and rotation of the confining cloud, but the walls lie outside the (roughly paraboloidal) surface within which a particle would have so little angular momentum that the hole could swallow it.

Collimation mechanisms influenced by non-newtonian gravitational effects near the hole have some peculiarly interesting consequences, discussed below. Such influences are restricted to length scales much smaller than those for which we have direct radio evidence. The beams, as they propagate outwards, cool in accordance with Bernoulli's equation; they may also cool radiatively, the resulting emission perhaps representing the non-thermal optical continuum from variable galactic nuclei¹⁵. Randomisation of bulk kinetic energy occurs in the components of extended double sources where the beams impact on an external medium³. Radiation from the beams themselves (and the superluminal compact sources in particular) indicates some dissipation along the beam due to shock formation, entrainment, or friction. The beam speeds may well remain relativistic out to distances of hundreds of kpc, at least in the stronger sources, otherwise the residual power input into the extended components would be much less than that dissipated by friction along the beam.

The common statement that the beams should be aligned with the associated galaxy's rotation axis (minor axis?) deserves more analysis. One should distinguish the rotation axes of the galaxy, of the infalling gas (which provides the fuel and influences the collimation), and of the black hole itself; anisotropies in the extragalactic environment may complicate the picture still more.

If the relevant gas comes from general stellar mass loss, and pervades (or has been accreted from) the whole galaxy, then its angular momentum vector should coincide with the galaxy's. The same statement holds for primordial gas falling into an elliptical: after one or two orbits its distribution would have become axisymmetric with respect to the galaxy³³. Thus, barring such complications as triaxiality³⁴, the gas should provide a stable long-term symmetry axis. But this gas may not provide an adequate fuelling rate for the most powerful sources³⁵ (unless it accumulates and is then dumped sporadically). This suggests that there may be an extra supply of gas near the nucleus. For instance, Hills^{20,21} invokes a 'debris cloud' of disrupted stars, in which the velocity dispersion is $v_* \approx 10^3 \text{ km s}^{-1}$. The storage time for gas in this cloud is $t_{\text{store}} \approx (r_s/c)(v_*/c)^{-3}$. The number of stars contributing to the cloud at any instant is then $N \approx t_{\text{store}} \dot{M}/m_*$; the angular momentum vector then jitters, on a timescale $\sim t_{\text{store}}$, by $\sim (N\varepsilon)^{-1} \text{ rad}$, ε being the oblateness of the central star cluster. This could be unacceptably large, suggesting the need for some other process to stabilise the beam orientation.

If the black hole is spinning, the Lense–Thirring 'dragging of inertial frames' influences material sufficiently close to it. An orbit of radius r and period t_{orb} that does not lie in the hole's equatorial plane precesses on a timescale $t_{\text{prec}} \approx t_{\text{orb}}(J/J_{\text{max}})^{-1}(r/r_s)^{3/2}$, J being the hole's angular momentum and J_{max} the maximum angular momentum of a Kerr hole. If the material drifts inwards only slowly (that is, $t_{\text{infall}}(r) \gg t_{\text{orb}}(r)$) then the precession builds up over many orbits. As Bardeen and Petterson³⁶ pointed out, there is then a critical radius r_{BP} at which $t_{\text{prec}} \approx t_{\text{infall}}$. For $r \leq r_{\text{BP}}$, the gas flow is axisymmetric with respect to the hole, irrespective of its original angular momentum. Thus, if the beams are collimated at a radius $\leq r_{\text{BP}}$, their orientation is, in effect, stabilised by the hole; they are then impervious to jitter resulting from short-term (timescale $\ll M/\dot{M}$) fluctuations in the flow pattern of the surrounding gas. If this is the reason for the extraordinary long-term stability of 3C236, the ratio of the size of the source to the dimensions of the nozzle must be $\geq 10^{10}:1$.

Black holes may generally develop from an accumulation of gas with the same provenance as that which later fuels the accretion process. We would then expect alignment of the hole's axis with the angular momentum of the galaxy. But one can envisage circumstances when a galaxy might acquire a misaligned black hole. Suppose, for example, that a galaxy harbouring a central hole—a relic of earlier violent activity—collided or merged with another galaxy³⁷. The new merged galaxy would then be misaligned with its central hole. (The same holds true if the merging galaxies each contained a black hole, and the two holes coalesced.)

What happens if nuclear activity in this merged galaxy is revived by capture of intergalactic gas?³³ Beams collimated at radii $\leq r_{BP}$ will emerge along the hole's rotation axis. But this axis will gradually swing as the accretion proceeds; owing to the Bardeen–Peterson³⁶ effect, each unit mass of infalling material changes the hole's angular momentum by $\sim J_{\max}(r_{BP}/r_s)^{1/2}$. The timescale for the hole to align with its new surroundings is thus $t_{BP} \approx (M/\dot{M})(J/J_{\max})(r_s/r_{BP})^{1/2}$. (This process is more rapid than any forced precession³⁶, unless the black hole acquires a charge which permits electromagnetic torques to act on it³⁸). The value of t_{BP} cannot be estimated precisely, but is of the same general order ($\sim 10^8$ yr) as the overall lifetime of active nuclei.

Reviewers of morphology^{6,39} have noted a class of sources—for example, NGC315, 3C47, 3C192 and perhaps Cygnus A—characterised by reflection symmetry: it seems from the radio contours as though the two symmetrical oppositely-directed beams have gradually swung or drifted over the source lifetime. Perhaps these radio galaxies are systems where a pre-existing central black hole is gradually being torqued into alignment with the angular momentum of newly supplied fuel that has reactivated the nucleus. The gas and plasma dynamics in these sources must be even more complex than in typical doubles. Each beam would be slightly skewed by the transverse drift, the cocoon surrounding it being smeared into a fan shape. (The existence of sharply curved beams in trail sources such as NGC1265 shows that instabilities are not catastrophic even when there is a strong transverse pressure gradient⁴⁰). This class of sources obviously holds clues to the collimation mechanism and beam dynamics; but they also have added interest if their distinctive morphology is indeed a manifestation of general relativistic effects near a black hole, the scale of the phenomenon amplified 10^{10} -fold by the plasma beams.

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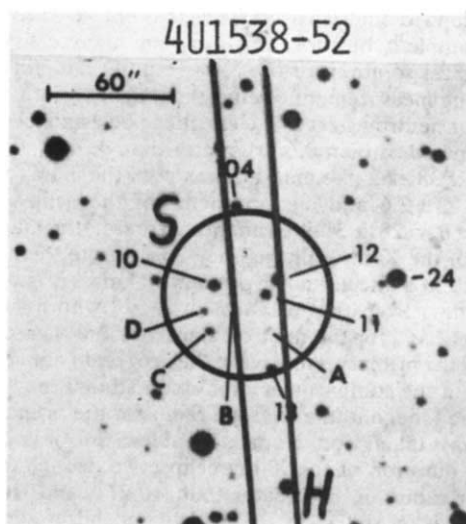
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Location of pulsating binary X-ray source 4U1538–52 with the HEAO-1 scanning modulation collimator

THE 529s pulsating^{1,2} X-ray source 4U1538–52 has been found to be a 3.73d eclipsing binary system³. The most accurate orbital elements yet determined⁴ imply that the primary is an early type giant or supergiant star. Independent determination of the primary mass will make it possible to estimate the mass of the (assumed) neutron star secondary. Cowley *et al.*⁵ have surveyed the stars in the Uhuru¹⁹ and Ariel 5⁴ error boxes and called attention to 10 stars consistent with the inferred properties of the primary. A precise (40") position of 4U1538–52 obtained by SAS 3⁶ includes only two of these stars⁵. This letter reports a HEAO-1 position measurement of precision 12" in one dimension which clearly selects one of these two stars (number 12) as the candidate identification.

The HEAO-1 observing programme allowed the scanning modulation collimator (MC) experiment^{7,8} to observe this region from 20 to 28 August 1977. Three constraints limited the data we could use in determining the position: the necessity to avoid the nearby source MX1608–52, which was in a strong transient state at this time^{9,10}; the lack of data during the X-ray eclipses; and the progressive deterioration and failure of one of the four MC2 (120" FWHM modulation collimator) proportional counters during this period. (The one counter was identified and turned off on 29 August 1977, and did not affect MC2 data subsequent to that date.) We therefore obtain only a

Fig. 1 Circle, SAS 3 90% confidence error region. Heavy lines, boundaries of 90% confidence error region determined by the 30 arc s modulation collimator. Numbered stars were observed by Cowley *et al.*⁵. Lettered stars appear in the finding chart given by Apparo *et al.*⁶.



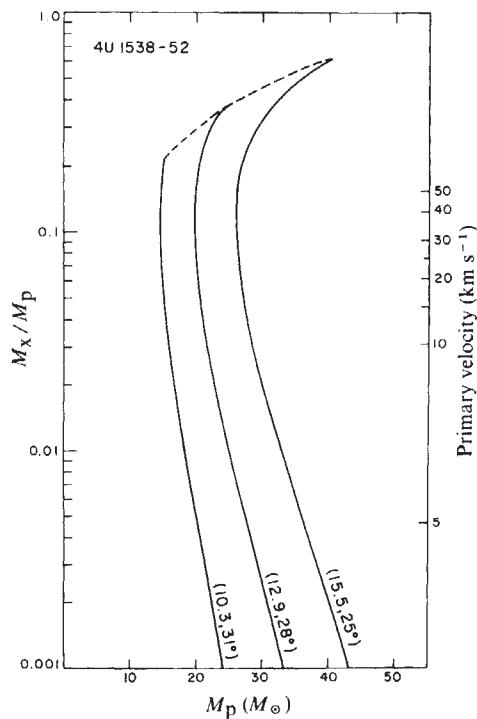


Fig. 2 Abscissa, mass of the primary in units of the solar mass. Ordinate: $q = M_x/M_p$ on left scale, K = orbital velocity of primary on right scale. Dashed curve: upper limit to allowed parameters if the inclination $i = 90^\circ$. The solid curves are denoted by (X-ray mass function, eclipse half-angle) and enclose the region allowed for $f_M = 12.9 \pm 2.6 M_\odot$ and $\theta_e = 28^\circ \pm 3^\circ$.

single line of position, using 12 orbits of data from MC1 (30" FWHM modulation collimator) on 25 August 1977. The half-width of the error region is $12''$ (90% confidence), considering counting statistics and aspect solution accuracy. We measure an average flux of $(1.2 \pm 0.2) \times 10^{-10} \text{ erg cm}^{-2} \text{ s}^{-1}$ (2–6 keV), with an additional systematic uncertainty of about 30%.

Figure 1 shows this line of position with the SAS 3 error circle. Stars 10 and 12 are among the candidates noted by Cowley *et al.*⁵. Of all the stars having m_i and $B - V$ consistent with an OB supergiant as required by the X-ray mass function^{3,4}, only star 12 is allowed by the present location. Preliminary optical reports indicate that star 12 is an early-type star¹¹ with He I and H α emission¹², and with radial velocity variations $K \sim 25 \text{ km s}^{-1}$ consistent with the X-ray orbital period, thus proving the identification¹³.

The present system is the fifth example of an X-ray pulsator with a well-defined binary period. The transient 4U0115 + 63 is the sixth example²⁰, but does not show an X-ray eclipse and therefore a mass solution for the X-ray emitter can not yet be made, pending measurement of ellipsoidal light variations. For the other four neutron stars as a class, it can be established that none has a mass less than 0.6 or greater than $4.0 M_\odot$ (refs 14, 15). For 4U1538–52, the measurements⁴ of the mass function give $f(M) = 13 \pm 2.6$ and measurements of the eclipse semi-duration give $\theta_e = 28^\circ \pm 3^\circ$. We can then express¹⁵ the mass ratio $q = M_x/M_p$ of the X-ray emitting (neutron) star to the primary star as a function of the unknown primary star mass (Fig. 2). This approach differs from that of Davison *et al.*⁴ who assumed a range of 1 to $2 M_\odot$ for the neutron star mass and then derived properties of the primary. Here we make no *a priori* assumptions on the mass of the compact star. It is assumed that the primary fills its Roche lobe, and the average radius of the primary as a function of q is taken from Kopal's¹⁷ Table 3-2.

From the duration of the X-ray eclipse we deduce that the primary star radius R_1 is greater than $10 R_\odot$, and from the semi-major axis⁴ $a_x \sin i = 55.2 \pm 3.7$ light-seconds we deduce¹⁷

$R_1 \leq 34 R_\odot$. These limits restrict the star to be either an early type (OB) or a red giant. However, red giants have masses $< 10 M_\odot$, and are directly excluded by the mass function value, as shown in Fig. 2.

For the present candidate, Cowley *et al.*⁵ give a measured $B - V = 2.1$ and apparent $V = 14.1$. Since $(B - V)_0 = -0.3$ for an OB supergiant¹⁶, we have $E_{B-V} = 2.4$ and $A_V = 3.2 E_{B-V} = 7.7$. Such a large reddening in this direction ($l = 327^\circ$, $b = 2.2^\circ$) implies a source located at least 2 kpc distant, behind the Sagittarius–Carina spiral arm. The absolute magnitude is then $M_V \leq -5.4$, and the bolometric luminosity $L_{\text{bol}} \geq 2 \times 10^{38} \text{ erg s}^{-1}$. If we equate $L_{\text{bol}} = \pi R_1^2 \sigma T^4$, we obtain effective temperatures of 1.5 to $3 \times 10^4 \text{ K}$, consistent with B spectral type¹², for the above range of primary star sizes.

From Fig. 2 the allowed range of values of the neutron star mass can be determined for any given primary star mass. For a B0 supergiant, we would expect $M_p \sim 35 M_\odot$ (ref. 22). This would imply $M_x/M_p < 5 \times 10^{-3}$, or $M_x < 0.18 M_\odot$. However, stars in close binary systems are typically less massive than indicated by the spectral type^{15,18,23}, and we could clearly have $M_p = 25$, and any $M_x \leq 10$. Crampton *et al.*²¹ have reported an optical velocity of $33 \pm 7 \text{ km s}^{-1}$. This formally gives $0.08 \leq q \leq 0.13$. From the X-ray mass function and eclipse duration as expressed in Fig. 2, we can derive corresponding limits of $1.2 \leq M_x \leq 3.3$. If the optical velocity were precisely 33 km s^{-1} , uncertainty in the X-ray mass function would allow $1.5 \leq M_x \leq 2.7 M_\odot$.

Determination of the masses of large numbers of neutron stars is of the utmost importance to discriminate among the upper limits to the mass allowed by various models of nuclear structure in the framework of general relativity. For the system 4U1538–52 we still need proof that the compact object is indeed a neutron star, for example, by observation of changes in the pulse period which may be attributed to accretion torques. Further optical study of star 12 is clearly needed to confirm the optical observation of the 3.73-d period, and to measure the optical mass function and ellipsoidal light variations as accurately as possible.

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Io may have a bright dawn terminator

NELSON AND HAPKE¹ have shown that occasional observations of post eclipse brightenings (PEB) of the planet Jupiter's satellite Io are very strongly positively correlated with the occurrence of major solar flares within 10° of the solar central meridian (as seen from Jupiter) during the 100 days before the eclipse observation. No eclipses which showed no brightening were preceded by such flares. Io is in the outer part of the jovian radiation belts. Energetic particle fluxes in the Earth's radiation belts at a roughly corresponding location are enhanced for roughly similar times (the 100 days in ref. 1) following the major geomagnetic storms caused by major flares near central meridian^{2,3}. The 100-day time scale is also in reasonable agreement with diffusion times in recent models of the jovian magnetosphere⁴. Considering all this, Nelson and Hapke inferred from their flare correlation that the occurrence of a noticeable PEB effect is due to enhanced fluxes of trapped energetic particles. They suggested that there may be a process similar to thermoluminescence, which converts some fraction of the particle dose received during the eclipse, into visible light which is emitted when the surface is again sunlit, and decays with the characteristic PEB time scale of ~10 min. Although Fanale *et al.*^{5,6} had already suggested that solid state effects in the presence of irradiation by energetic particles might play a part in the PEB phenomenon, the particular correlation with solar flares that Nelson and Hapke found points at once to an energetic particle effect because that is what would be expected to be enhanced by such flares, and at the same time apparently explains the erratic occurrence of PEB on Io and its absence on more distant satellites (because they see much lower fluxes of trapped particles, enhanced or not). In any case, Nelson and Hapke have made a novel yet plausible suggestion which can be tested by laboratory simulation experiments of the solid state process, by more eclipse observations from Earth, and by observations of jovian trapped particles by future flyby and orbiter spacecrafts, and their relation to solar flares. This note suggests another particular test of the thermoluminescence properties of Io which can be made with the unique optical observations which are programmed for the close Voyager I Io flyby in March 1979, and which does not necessarily require a previous suitable flare, as the PEB phenomenon seems to do.

If Io's surface is thermoluminous with the characteristic PEB time scale of ~10 min, there should be a characteristic bright rim on the sunlit side of Io's dawn terminator. This is essentially because each point on the surface has been 'eclipsed' by Io itself during the night, which is 10 times longer than eclipse, and should accumulate particle dose during the night just as the whole surface would do when eclipsed by the planet. At dawn, it must release all this dose as light on the 10-min time scale. If the time constant in the dark is fairly long compared to the 21-h Io

night, the dawn terminator will be 10 times brighter per unit particle flux than the PEB effect.

To illustrate this, consider a model in which D = stored dose, erg cm^{-2} ; K = light emission rate per stored dose = $K_L \sim 0.1 \text{ min}^{-1}$ in sunlight = K_D ($\ll K_L$) in the dark; e = efficiency of the process; F = particle dose rate, $\text{erg cm}^{-2} \text{ sec}^{-1}$; L = luminosity = KD ; t_e = duration of eclipse. Then, both in sunlight and in the dark,

$$\frac{dD}{dt} = -KD + eF \quad (1)$$

After a few times 10 min in sunlight, the luminosity reaches its equilibrium value $L = eF$. During eclipse or during the night, taking $t = 0$ at immersion

$$L = (K_D/K_L)eF \exp(-K_D t) + eF(1 - \exp(-K_D t)) \quad (2)$$

After eclipse, or at dawn (now taking t as the local Io time since the Sun re-appeared),

$$L = (K_L/K_D - 1)(1 - \exp(-K_D t_e))eF \exp(-K_L t) + eF \quad (3)$$

the spatial shape of the bright dawn terminator in this model is given by replacing t in equation (3) by Φ/Ω , where Φ is longitude from the Io dawn terminator, and Ω is the rotation rate $2\pi/1.79$ days. If $K_D t_e \ll 1$, the luminosity immediately after eclipse or dawn, is

$$L_B \sim (1 + K_L t_e)eF \quad (4)$$

This is about 15 eF for jovian eclipses and about 130 eF for the bright dawn terminator effect. The light curve predicted by equations (2) and (3) with $K_D t_e \ll 0.1$ is shown in Fig. 1.

There are two areas where the idea of bright dawn terminator suggested here is related to the Nelson-Hapke model of PEB. (1) The size and shape of the bright region near the dawn terminator depends in detail on the parameters in equation (3). If one supposes that thermoluminescence occurs on Io without specifying the rate constants, a distinct small bright area is not generally predicted. It occurs in our model because we use the PEB decay rate of 0.1 min^{-1} for K_L . (2) The actual luminosity of the bright area near the terminator is predicted by equation (4) to be 130/15 times the brightness of PEB at the same particle dose rate. Turning this around, one concludes that if it is fairly sure that the dose rate enhancements induced at Io by solar flares are not much more than a factor of 130/15, the bright dawn terminator should be at least as bright as the PEB.

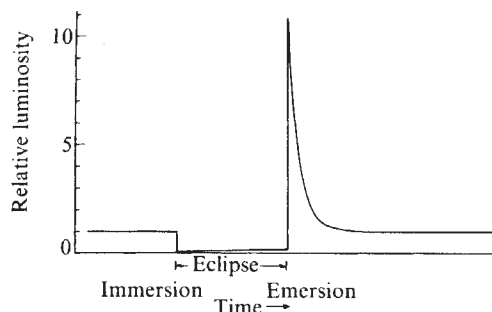
The important point about the predicted bright dawn terminator is that it would provide an opportunity to observe the colour and temperature dependence of the suggested Io thermoluminescence whenever the flyby spacecraft arrive, since it does not require an eclipse and may not require enhanced particle fluxes.

The bright dawn terminator should extend about 10 min of the Io day from the geometrical terminator in our model. This is only $(10 \text{ min}/42 \text{ h}) \times 360^\circ = 1.4^\circ$ of Io longitude into the sunlit side. It is not surprising this has never been observed from Earth, as it is small and highly foreshortened.

On 5 March 1979, Voyager I will observe Io optically from altitudes of 22,000 to 65,000 km, including large areas around both the dawn and dusk terminators within about 60° of Io's south pole, in six spectral regions defined by colour filters from violet to yellow (E. C. Stone, personal communication). The spatial resolution of these pictures should be adequate to reveal the bright dawn terminator effect if it exists and is bright enough.

The shape of the predicted bright area near Io's dawn terminator is actually determined by how K_L (see, for example, ref. 1) really changes locally as the Sun rises. Thus, the shape of the bright regions can be used to determine the variation of K_L and will be an important constraint on the thermoluminescence model. The eclipse light-curves are not sensitive to this effect. For example, if K_L varies after dawn like $K_L \sin \Omega t$ where Ω is the rotation frequency of Io and t the time since dawn, the brightness of the terminator is reduced to about $eF (K_L t_e)^{1/2}$ and

Fig. 1 Possible light curve of 'thermoluminescent' light from Io with parameters given in the text. If the thermoluminescent emission rate is proportional to the solar illumination, the bright dawn terminator effect is moved to the right and its intensity reduced.



the position of maximum intensity is pushed away from the geometrical terminator, to about $(\pi K_L t_e)^{1/2}$ radians, or 9° of longitude into the dayside of the terminator. Thus it is quite important that we have adequate resolution in Io longitude to determine the position of the maximum brightness, and other details of the spatial dependence of this effect, so that the intensity of the effect may be properly interpreted. Particle flux levels measured at the same time will determine e . The colour of the excess light should help to define the 'thermoluminescent' system. Comparison of the planned observations of the terminator 1 h apart (E. C. Stone, personal communication) will determine some spatial variations in e and possibly other aspects of this process which might well occur due to uneven bombardment of Io by the corotating energetic particle fluxes in the jovian magnetosphere.

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Millisecond microwave spikes in a solar flare

THE shortest-lived structures in microwave solar flares known until now last ~ 1 s. Recently (11 April 1978) we observed a microwave outburst at 2.65 GHz (starting at 13.41 UT) showing a strong and extremely fast fluctuating burst component. Many spikes appeared to have half-power durations smaller than the 20-ms resolution of the instrument used. These are about two orders of magnitude shorter-lived than events reported from previous microwave observations. They are only rivalled by fine structure in some decimetric type IV bursts between 0.4 and 1.4 GHz (ref. 1).

The occurrence of microwave flares with millisecond fine structure is probably not very rare as we observed a second example on 28 April 1978 around 13.50 UT. Our resolution at

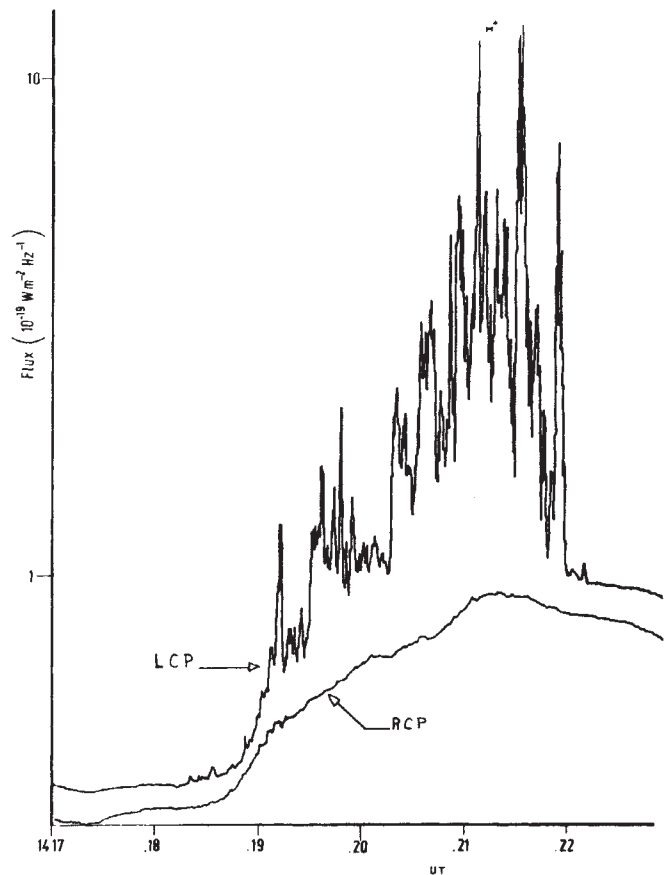
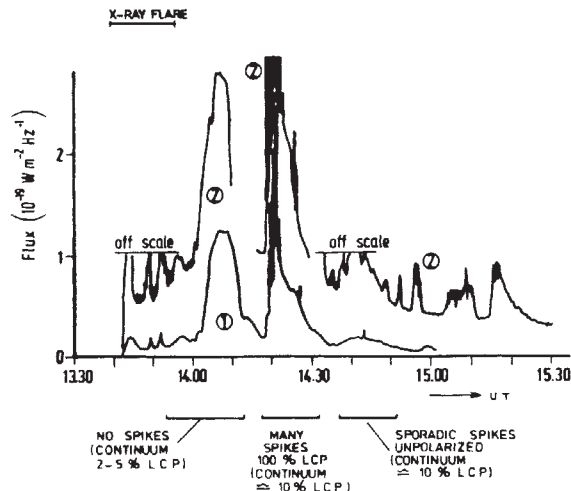


Fig. 2 Flux density of both left (LCP) and right (RCP) circularly polarised components of the most variable part of the event. The fine structure is not resolved. The indicated fragment (*) of about 2 s is expanded in Fig. 3. Note that this is not one of the most intense peaks but rather a typical one.

that time was limited to 100 ms, which turned out to be insufficient. The importance of this last flare in H_α was rather high, 4B, but that of the flare at 11 April had the more common rating 2B.

Our observations were made with a double sideband receiver sensitive to two bands of 12 MHz separated by 60 MHz. The receiver measures both left and right circularly polarised radiation (LCP and RCP), with an effective time constant of 10 ms. The outputs are recorded on stripchart recorders, allowing a maximum resolution of 100 ms, and, for full resolution, on magnetic tape with a sampling interval of 10 ms.

The microwave flare consisted of a large number of successive bursts (see Fig. 1). Some of these showed millisecond structure. The general behaviour on 2,650 MHz (Dwingeloo) corresponds very well with that on 2,800 MHz (Ottawa), but a sharp high frequency cut off is quite clear during the whole event. For the smooth component we found a fall of 3-5 dB over only 150 MHz. The fluctuating component is less easy to compare as the Ottawa time constant is much larger than the duration of the spikes. Comparison of this component after averaging over 0.5 s in both recordings yields a fall of 5-7 dB. The three major bursts (14.05, 14.20 and 14.40 UT) showed at 2,650 MHz a weakly L

Fig. 1 Reconstruction for H_α flare, 2B, N19W54, of the total power recording of 2,650 MHz flux observed at Dwingeloo (2) compared with that of 2,800 MHz flux observed at A.R.O. (1) (courtesy M. B. Bell at A.R.O., Ottawa). Data reconstructed from a logarithmic channel. The period of strong variability coincides for both frequencies. This period is shown expanded in Fig. 2.

polarised continuum, the first one without any spikes, the second one with a very large number of 100% L.C.P. spikes and the last one with sporadic, hardly (if at all) polarised, spikes (one might consider the weakly polarised continuum to be composed of spatially different L and R sources, but then these would have to show quite precisely the same temporal development; besides the occurrence of weakly polarised spikes sustains the concept of a weakly polarised source).

The spikes around 14.21 UT were analysed in detail. All were 100% L.C.P. Flux densities of up to $6 \times 10^{-19} \text{ W m}^{-2} \text{ Hz}^{-1}$ were observed for the spikes for nearly all durations (Fig. 3). The occurrence of these spikes starts along with the rise of the continuum, but ends rather abruptly (Fig. 2) long before the continuum died out.

The half-power durations of the spikes were almost all (95%) below 40 ms, while the abundance increased with decreasing duration (Fig. 4). This implies that many spikes were not resolved. The intervals between successive spikes were also rather short: 72% were below 50 ms and again their abundance was larger the shorter their value. There were no conspicuous periodicities but too many values were rather close to the sampling interval.

An important consequence is that the particle acceleration mechanism must be effective on the time scale of milliseconds, which is 2 or 3 orders of magnitude shorter than hitherto envisaged in most models^{2,3}. However, recently a pulsed

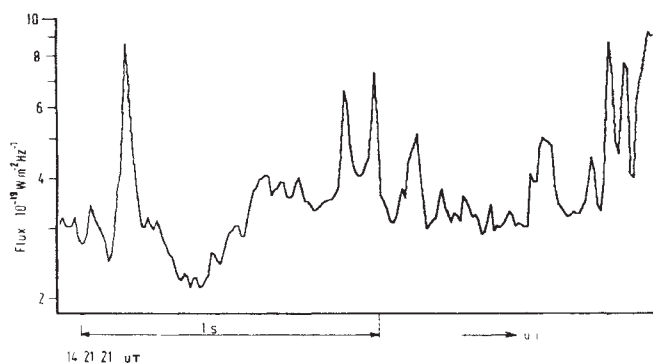


Fig. 3 Full resolution recording of a typical fragment of the fine structure. The intervals between measured flux values are 10 ms. Date, 11 April 1978.

acceleration process has been proposed⁴ where the time scales of the acceleration is the local collision time. Indeed the collision damping rate for plausible source conditions (kinetic temperature 10^7 K , particle density 10^{11} cm^{-3}) is a few milliseconds. Hence we assume the spike durations to be determined by the excitation periods. We can now estimate the source dimensions by assuming a spatial growth rate between the Alfvén speed and c . A plausible Alfvén speed would be $\approx 3,000 \text{ km s}^{-1}$ (field strength $\approx 300 \text{ G}$). A spike lasting 10 ms would then have a source width between 30 and 3,000 km. As an m.h.d. instability is the most likely generating process^{2,3}, dimensions of the order of tens of kilometres are a realistic estimate. Such values are considerably shorter than observed for microwave sources ($\approx 8,000 \text{ km}$)³ and H_α kernels (3,000–6,000 km), but have been hinted at in theoretical models^{2,3}.

The flux densities of the spikes, together with the source size estimates given above, lead to a lower limit of the brightness temperatures of $\sim 10^{15} \text{ K}$. Obviously a coherent radiation mechanism is necessary to account for such values. It is interesting to note that the completely polarised spikes can be explained as plasma radiation at the fundamental frequency, arising from induced scattering of Langmuir waves present in the pulsed acceleration model⁴, while the continuum burst can be explained as weakly polarised self-absorbed gyro-synch-

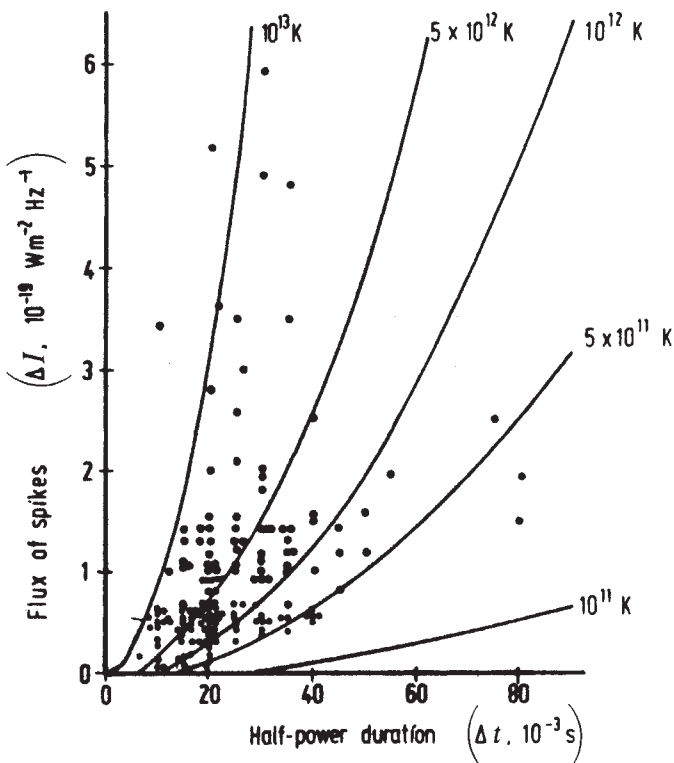


Fig. 4 Peak flux values of spikes plotted against their half-power durations. Curves of constant brightness temperature are drawn taking the Alfvén speed times the duration as typical dimension of the spike sources. Durations below 10 ms are cut off by the time constant of the receiver.

rotron radiation from the heated and accelerated particles all over the flare region. Observation of microwave spikes should provide direct evidence of the elementary flare processes.

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First mass analysis of stratospheric negative ions

ATMOSPHERIC negative ions have been identified previously by *in situ* mass spectrometric measurements only at altitudes above $\sim 70 \text{ km}$, which was the operational height limit of the rocket-borne instruments used^{1,2}. These measurements revealed the presence of atomic, molecular and cluster ions mostly having masses between 35 and 152 AMU. Here we report preliminary results of the first mass spectrometric measurements of stratospheric negative ions. Only observed mass numbers will be presented. A detailed analysis of the ion abundances, will be published elsewhere.

Three flights of a balloon-borne mass spectrometer probe were conducted during daytime over southwestern France (flight 1: 2 September 1977; flight 2: 26 September 1977; flight 3: 10 November 1977). Peak altitudes of 33.5, 35, and 37 km were reached. The instrument, developed and built at the Max-Planck-Institut für Kernphysik, has been previously described along with the circumstances of the flight measurements and the preliminary positive ion composition data obtained simultaneously³ and will not be reported here. Note, however, that the mass resolution setting of the instrument was lower for negative ions (~ 22) than for positive ions (~ 50) in favour of an increased transmission of the mass filter. Thereby a signal-to-noise ratio for negative ions comparable to that for positive ions should be achieved considering the higher dark count rate of the ion detection system in the negative ion measurement associated with the higher voltage applied to the channeltron.

Most negative ion data were obtained at the peak altitudes and only these will be reported here. As far as the major masses are concerned, the data obtained in the different flights agree with each other. The uncertainty of the mass identification up to ± 3 AMU is due to the limited definition of the flanks of the mass peaks. Laboratory and in-flight-calibrations of the mass scale were performed³. The width of a mass peak is typically about 15 AMU which implies that small mass peaks may easily become covered by large ones and thus may not be detectable.

The major ion masses (fractional abundance $> 1\%$) given in Table 1 cover the range from 125 to 295 AMU. When compared to the masses of the major positive ions observed in the same flights ranging from 55 to 140 ± 2 AMU (ref. 3), they are larger and are also greater than the negative ion masses observed above 70 km which were mentioned before.

Table 1 Mass numbers and tentative identifications of major negative ions observed at 33–37 km altitude

Mass	Tentative identification
125 $\pm$ 2	$\text{NO}_3^-\text{HNO}_3$
161 $\pm$ 2	R^-HNO_3 $\text{NO}_3^-\text{HNO}_3\text{HCl}$
188 $\pm$ 2	$\text{NO}_3^-(\text{HNO}_3)_2$
197 $\pm$ 3	R^-HR
224 $\pm$ 3	$\text{R}^-(\text{HNO}_3)_2$ $\text{NO}_3^-(\text{HNO}_3)_2\text{HCl}$
253 $\pm$ 3	$\text{NO}_3^-(\text{HNO}_3)_3$
260 $\pm$ 3	R^-HRHNO_3
289 $\pm$ 3	$\text{R}^-(\text{HNO}_3)_3$ $\text{NO}_3^-(\text{HNO}_3)_3\text{HCl}$
295 $\pm$ 3	$\text{R}^-(\text{HR})_2$

For identification of R see text.

An identification of the chemical nature of the observed negative ions is difficult at present mainly because of the limited mass accuracy, the lack of rate constants of relevant ion molecule reactions and the poor knowledge of stratospheric trace gases. Nevertheless, some general conclusions concerning the nature of the observed ions may be drawn.

The high mass numbers probably indicate cluster ions consisting of a core ion and attached ligand molecules. An upper mass limit of the least massive core ion is defined by the smallest major ion mass (125) observed. This is consistent with a core ion NO_3^- (62 AMU) being considered as the favoured core ion of stratospheric negative cluster ions because of the large electron affinity of NO_3^- (ref. 4). If the core ion is actually NO_3^- , mass 125 may be identified as $\text{NO}_3^-\text{HNO}_3$. The average mass of the building blocks of the cluster ions (core ion and ligand molecules) may be estimated by considering the following argument: the association order (number of ligand molecules attached to the core ion) of the negative cluster ions should be less than that of the $\text{H}_3\text{O}^+(\text{H}_2\text{O})_n$ ions measured simultaneously. This has to be expected as the atmospheric concentration of the ligand molecules is probably lower than that of water and since the

bond energies of negative cluster ions inferred from laboratory measurements is generally lower than that of the $\text{H}_3\text{O}^+(\text{H}_2\text{O})_n$ ions of the same association order (see ref. 5). A lower limit for the mass of the building blocks of about 74 ± 3 AMU may be estimated by considering a maximum association order of 3, the same as that of the observed $\text{H}_3\text{O}^+(\text{H}_2\text{O})_n$ ions and a maximum mass of the observed major negative ions of 295 ± 3 AMU. A large average mass of the ligand molecules is also inferred from the large mass difference of adjacent mass peaks. This rules out an identification of the observed negative ions as hydrates which were previously considered to dominate in the stratosphere⁶.

Our conclusion is supported by laboratory measurements of gas phase hydration equilibria of negative ions (see refs 7, 8; Fehsenfeld, personal communication; ref. 8) inferring that negative hydrates should contain only one or two water molecules at water vapour pressures and temperatures prevailing at 34–37 km altitude. Most likely, the ligand molecules are stratospheric trace gases which are much less abundant and which are stronger bound to negative ions than water.

The ligand molecules should have large gas phase acidities as laboratory studies indicate that the bond energy of a molecule to a negative ion increases with the gas phase acidity of the molecules⁹. Favoured candidates therefore seem to be, HI , HNO_3 , HBr , HCl , and H_2SO_4 . Cluster ions of the type $\text{NO}_3^-(\text{HNO}_3)_n$ which were previously proposed to exist in the stratosphere⁴ would be consistent with the observed masses 125 ± 2 ($\text{NO}_3^-\text{HNO}_3$), 188 ± 2 ($\text{NO}_3^-(\text{HNO}_3)_2$) and 252 ± 3 ($\text{NO}_3^-(\text{HNO}_3)_3$). The observed masses 161 ± 3 , 197 ± 3 , 224 ± 3 , 260 ± 3 , 289 ± 3 and 295 ± 3 seem to fit a pattern $\text{R}^-(\text{HR})_m(\text{HNO}_3)_n$ with HR having a mass of 98 ± 2 AMU and with $m + n \leq 3$ (Table 1). A tentative candidate for HR seems to be H_2SO_4 . Another possibility might be HClO_4 but this is unlikely to be sufficiently abundant in the upper stratosphere.

The identification of the observed ions deserves further investigation involving the determination of relative ion abundances, *in situ* measurements with higher mass resolution and laboratory studies of relevant ion reactions. Such measurements should also provide a base for an analytical application of stratospheric ion composition measurements in terms of a determination of the concentrations of certain neutral trace gases involved in stratospheric ion molecule reactions. If the observed ions would actually contain HNO_3 , H_2SO_4 and HCl , it should be possible to derive the concentrations of these gases from the ion composition. Thus, ion composition measurements may become a powerful analytical tool, which allows the detection of very small gas concentrations^{3,4,10}.

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Effect of the reaction $\text{HO}_2 + \text{O}_3 \rightarrow \text{OH} + 2\text{O}_2$ on stratospheric ozone

THE recent measurements¹⁻³ of large values (8.1 ± 1.5 and $8.2 \pm 2.7 \times 10^{-12} \text{ cm}^3 \text{ s}^{-1}$) of the rate coefficient for the reaction



have led to some important revisions in our understanding of stratospheric photochemistry⁴. In particular, the predicted ozone perturbations due to the injection of nitrogen oxides by supersonic transport aircraft have become net ozone gains instead of losses⁵, while the predicted ozone depletion due to ozone-active chlorine from chlorofluoromethanes has almost doubled⁴. An unfavourable result of the adoption of the larger value of the rate coefficient for reaction (1) has been the prediction of excessive amounts of stratospheric ozone (10–20% more than the measured concentrations) both in terms of the total column abundance and the concentration profile at altitudes below 40 km. Attempts to remedy this discrepancy between theory and observation by altering the simulated lower stratosphere transport and by increasing the assumed amount of ozone-active chlorine were not successful. It is the purpose of this letter, however, to show that another recent measurement by Zahniser and Howard⁶ of the rate coefficient, k_2 , for the reaction

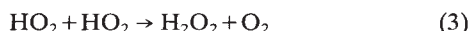


largely resolves these problems of excessive stratospheric ozone in models. Zahniser and Howard have reported the first direct measurement of the reaction between HO_2 and ozone (reaction 2). Their temperature-dependent rate coefficient is

$$k_2 = (1.4 \pm 0.4) \times 10^{-14} \exp [-(580 \pm 100)/T] \text{ cm}^3 \text{ s}^{-1}$$

which at stratospheric temperatures is 4 to 5 times larger than the value recommended in the report of the NASA Chlorofluoromethane (CFM) Workshop⁴, that is, $7.3 \times 10^{-14} \exp (-1,275/T) \text{ cm}^3 \text{ s}^{-1}$.

Before the work of Zahniser and Howard⁶ the only measurements of the rate coefficient for reaction (2) were the indirect competitive ones of Simonaitis and Heicklen⁷ and DeMore and Tschuikow-Roux⁸, who measured the ratio $R = k_2/(k_3)^{1/2}$ where k_3 is the rate coefficient for the reaction



The measured ratios are presented in Table 1. To estimate the rate for reaction (2) from these ratios, one must have information about the value of k_3 . For example, the NASA CFM Workshop report⁴ recommended a temperature independent value for k_3 of $2.5 \times 10^{-12} \text{ cm}^3 \text{ s}^{-1}$ with an estimated uncertainty of a factor of 2 for reaction (3). This rate constant was based on the simultaneous consideration of the best available rate data for several related hydrogen radical reactions⁴. Cox⁹, however, has since reported a large negative activation energy for reaction (3); his measured rate constant is $(1.4 \pm 0.7) \times 10^{-14} \exp [(1,650 \pm 205)/T] \text{ cm}^3 \text{ s}^{-1}$. Based on the newest data collected for reaction (3) and the R values of Simonaitis and Heicklen⁷ and DeMore and Tschuikow-Roux⁸, we can infer a much less marked temperature dependence for reaction (2) than was recommended in the CFM Workshop report⁴. Moreover, Hamilton *et al.*¹⁰ have found that the HO_2 radical involved in reaction (3) can readily cluster with water molecules—substantially accelerating the apparent rate of the reaction. Therefore the laboratory results obtained for R are expected to be complicated by HO_2 cluster reactions. Table 1 combines the kinetic data uncertainties for reaction (3) to estimate the 220 K (stratospheric) rate coefficients corresponding to each of the sets of available laboratory data. Note that the range of values of k_2 in Table 1 based on the measurements of Zahniser and Howard⁶

Table 1 Stratospheric $\text{HO}_2 + \text{O}_3$ reaction rate coefficient ($\text{cm}^3 \text{ s}^{-1}$)

Data source	$R = k_2/(k_3)^{1/2}$	k_2 (220 K) upper limit	k_2 (220 K) lower limit
Ref. 6	—	2.0×10^{-15}	4.5×10^{-16}
Ref. 4	—	4.4×10^{-16}	1.1×10^{-16}
Ref. 7	$1.2 \times 10^{-8} \exp (-1,000/T)$	$2.8 \times 10^{-16*}$	$1.4 \times 10^{-16*}$
		$1.3 \times 10^{-15\dagger}$	$2.8 \times 10^{-16\dagger}$
Ref. 8	$1.1 \times 10^{-7} \exp [-(1,550 \pm 250)/T]$	$6.7 \times 10^{-16*}$	$3.4 \times 10^{-17*}$
		$2.9 \times 10^{-15\dagger}$	$6.9 \times 10^{-17\dagger}$

* Based on the CFM Workshop⁴ recommended value for the rate coefficient for the reaction $\text{HO}_2 + \text{HO}_2$ of $2.5 \times 10^{-12} \text{ cm}^3 \text{ s}^{-1}$ ± factor of 2.

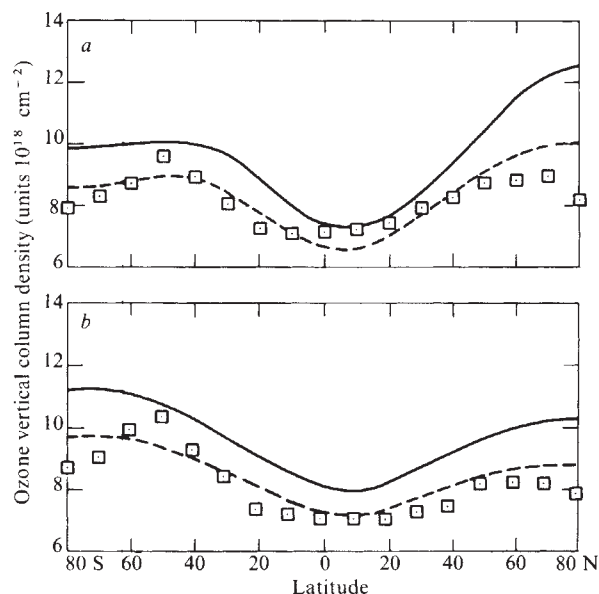
† Based on Cox's⁹ rate coefficient for the reaction $\text{HO}_2 + \text{HO}_2$ of $(1.4 \pm 0.7) \times 10^{-14} \exp [(1,650 \pm 205)/T] \text{ cm}^3 \text{ s}^{-1}$.

roughly coincides with the range of values deduced from the ratio measurements when Cox's effective rate for reaction (3) is adopted. In this sense, the previous measurements support the new, larger value for the rate coefficient k_2 .

We use a two-dimensional model of stratospheric trace constituents for our calculations^{11,12}. The model lies in a meridional plane extending from 80° N to 80° S latitude and from the Earth's surface to 60 km altitude. It computes the concentrations of 35 constituents and simulates meridional and vertical transport by seasonally variable large scale bulk motion and eddies. Except for reaction (2), the chemical rate coefficients employed are those recommended in the NASA CFM Workshop report⁴.

Figure 1 compares the measured latitude-dependent total ozone column densities in summer (a) and in autumn (b) in the Northern Hemisphere¹³ with the corresponding computed densities for both the old⁴ and new⁶ rate coefficients for reaction (2). Figure 2 shows two of the corresponding vertical concentration profiles of ozone at 0° and 45° N latitude in autumn for these two rate coefficient values of reaction (2). Note that the agreement between theory and measurement has improved more at high latitudes than at low latitudes. This latitude

Fig. 1 Total vertical column densities for a, summer and b, autumn in the Northern Hemisphere. The solid lines correspond to the CFM Workshop⁴ recommended value of the rate coefficient for the reaction of HO_2 with O_3 . The broken lines correspond to the rate coefficient reported by Zahniser and Howard⁶. The squares represent mean seasonal measured values collated by Wilcox *et al.*¹³.



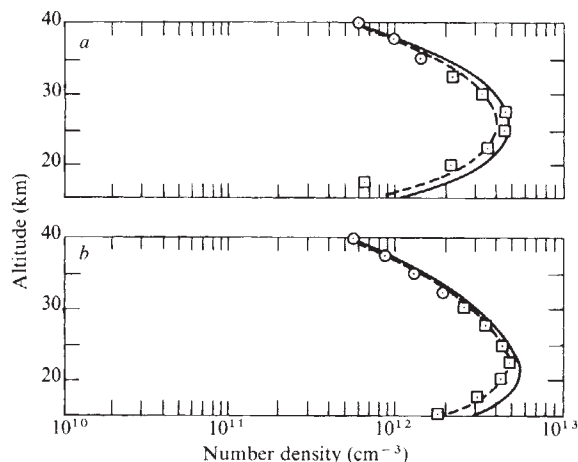


Fig. 2 Computed and measured ozone concentration profiles at latitudes of *a*, 0° and *b*, 40° N for autumn in the Northern Hemisphere. The squares represent the mean seasonal measured values collated by Wilcox *et al.*¹³. The Goddard data (circles) (I. Eberstein, personal communication) were obtained from satellite measurements of backscattered solar ultraviolet radiation¹⁴. The solid lines correspond to the CFM Workshop⁴ recommended value of the rate coefficient of the reaction of HO₂ with O₃ and the broken lines to the value reported by Zahniser and Howard⁶.

difference occurs because reaction (2) represents a larger fraction of the total ozone loss rate at the higher latitudes.

Reaction (2) represents the dominant catalytic loss process for ozone below 30 km. The dominance of this reaction over NO_x catalytic reactions results mainly from the new reaction rate coefficient for reaction (1). Poppoff *et al.*⁵ discuss the details of the coupled HO_x-NO_x photochemical cycles in the stratosphere for updated chemistry. They also give a sensitivity analysis (of total ozone to the injection of NO_x by supersonic transport aircraft) which is relevant to a study of the effects caused by changes in the HO₂ + O₃ reaction rate constant. Basically, since HO_x chemistry exerts strong control over the ozone abundance in the lower stratosphere, increasing the strength of the HO_x catalytic cycle [by increasing the rate constant for reaction (2)] tends to decrease the stratospheric ozone content.

Thus, we have found that the problem presented by the model predictions of excessive ozone abundances, which occurs when the recently measured value of the rate coefficient for reaction (1) is used, diminished significantly when the newly measured value of the rate coefficient for reaction (2) reported by Zahniser and Howard⁶ was used.

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Effect of pressure on the Raman spectrum of ice

THE decrease in the volume of ice on compression or cooling is thought to be due to a decrease in the O-O distances, because the O-O-O and O-H...O angles do not change. The decrease in the IR or Raman frequency of the in-phase O-H stretching vibrations, ν_1 , of ice is thus related to the consequent shortening of the hydrogen bond. It follows that ν_1 should remain constant at a constant volume, if anharmonic effects and the bending of O-H...O angles are insignificant. We report here the effect of pressure on the Raman spectral region of O-H stretching in hexagonal ice and discuss it in terms of explicit temperature and volume effects.

A cylindrical steel vessel with three 1-cm thick sapphire windows was used as the high pressure optical cell. The 5-mm diameter, 20-mm long sample of polycrystalline ice was mounted on the inside of one sapphire window with the cylindrical axis along the direction of the laser beam of power level 700-800 mW of the 4,880-Å excitation line. The optical cell was filled with Dow-Corning silicone oil, which acted as the pressure-transmitting fluid. The scattered light was collected at 90° to the laser beam and its intensity was measured with a calibrated Jarrel-Ash model 25-400 laser Raman spectrometer using a spectral slit width of 7-8 cm⁻¹.

Typical Raman spectra in the O-H stretching region (2,800-3,600 cm⁻¹) are given in Fig. 1. The features of the spectrum at high temperatures are relatively broad, because of the increase in the distribution of energy states, but they are essentially similar to those in the spectra measured below 100 K in our own study, or in the literature^{1,2}. In Fig. 1, the spectral features of ice remain unaffected by the presence of silicone oil, but two additional peaks appear at about 2,904 and 2,966 cm⁻¹ due to the C-H stretching mode in silicone. Application of pressure to ice at 255 ± 0.3 K decreases the frequency of the O-H stretching peak from 3,145 cm⁻¹ at 1 bar to 3,127 cm⁻¹ at 1.25 kbar.

A plot of ν_1 against pressure P at 255.2 ± 0.3 K is shown in Fig. 2. The $(\partial\nu_1/\partial P)_T = -13.7$ cm⁻¹ kbar⁻¹, a value which shows little systematic dependence on temperature in the region 269 > T > 253 K. A plot of ν_1 against temperature at 1 bar is also shown in Fig. 2. The $(\partial\nu_1/\partial T)_P = 0.63$ cm⁻¹ K⁻¹.

The effect of temperature on ν_1 at constant pressure may be divided into the constant-volume temperature effect, $(\partial\nu_1/\partial T)_V$, and the constant-temperature volume effect, $(\partial\nu_1/\partial V)_T$ by the equation

$$(\partial\nu_1/\partial T)_P = (\partial\nu_1/\partial T)_V + (\partial\nu_1/\partial V)_T(\partial V/\partial T) \quad (1)$$

$$(\partial\nu_1/\partial V)_T(\partial V/\partial T) = -(\alpha/\beta)(\partial\nu_1/\partial P)_T \quad (2)$$

where α is the volume expansivity and β the compressibility. At 255 K, $\alpha = 155$ MK⁻¹ (ref. 3) and $\beta = 12 \pm 1$ Mbar⁻¹ (ref. 4). From Fig. 2, $(\partial\nu_1/\partial P)_T = -13.7$ cm⁻¹ kbar⁻¹, and $(\partial\nu_1/\partial T)_P = 0.63$ cm⁻¹ K⁻¹. Substituting in equations 1 and 2, one obtains $(\partial\nu_1/\partial T)_V = 0.45$ cm⁻¹ K⁻¹. Thus the change in the O-H stretching frequency of ice with temperature near 255 K is about 30% due to the change in volume with temperature and about 70% due to the change in temperature alone. Thus the Grüneisen constant for the O-H stretching vibrations, $\gamma = -(\partial \ln \nu_1 / \partial \ln V)$, calculated from the above values is -0.36 at 255 K.

From the compressibility of ice⁴, the change in O-O distance with pressure, $(\partial R_{O-O}/\partial P)_T = -9$ Å Mbar⁻¹ at 255 K, where R_{O-O} is the O-O distance. From the R_{O-O} data⁵ adjusted to the

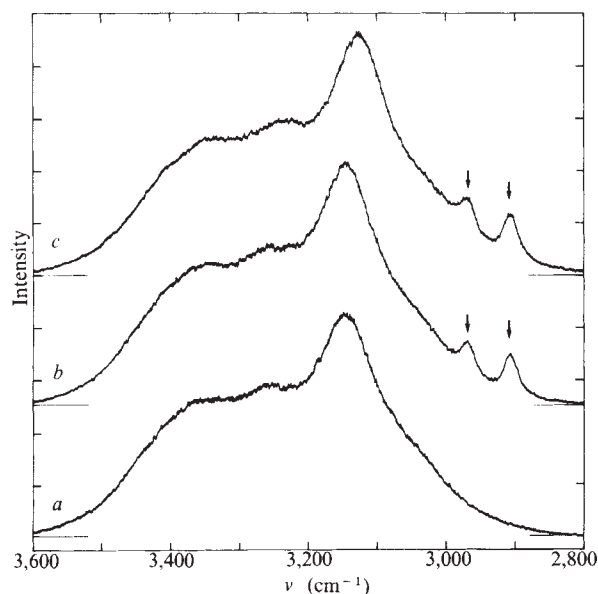


Fig. 1 Raman spectrum of hexagonal ice at 258 ± 0.2 K in the O-H stretching region. *a*, Ice held in air; *b*, immersed in silicone oil at 1 bar; and *c*, immersed in silicone oil at 1.25 kbar. Arrows point to the frequencies of the C-H stretching in silicone. Note also the slight increase in the C-H stretching frequencies with pressure.

density calculated from crystallographically determined expansivity³ of ice, the change in R_{O-O} with temperature, $(\partial R_{O-O}/\partial T)_P = 116 \text{ \AA MK}^{-1}$ at 255 K and 1 bar. Combining them with the values of the slope of the plots in Fig. 2, one obtains $(\partial \nu_1/\partial R_{O-O})_P = 5,430 \text{ cm}^{-1} \text{ \AA}^{-1}$ and $(\partial \nu_1/\partial R_{O-O})_T = 1,520 \text{ cm}^{-1} \text{ \AA}^{-1}$. So, for the same amount of decrease in O-O distance, isobaric cooling decreases ν_1 3.5 times more than does isothermal compression near 255 K.

The increase in ν_1 with temperature at a constant volume (or constant R_{O-O}) is of principal interest. The relatively high

positive value of $(\partial \nu_1/\partial T)_V$ indicates either or all of the following three: (1) the lengthening of the hydrogen bond with increasing temperature; (2) an unusual effect due to the anharmonicity of the potential energy of O-H stretching; (3) the weakening of the intermolecular coupling of O-H stretching motions, when the O-O distance remains constant. The lengthening of the hydrogen bond can occur either by the bending of O-H...O angles or (but less likely) by a shortening of O-H distance if the O-H...O bond were to remain linear. The force constant for the O-H...O bending in ice is small⁶ and the IR frequencies of librational modes indicate that the energy required to bend the O-H...O angle becomes smaller as the temperature increases. If lengthening of the hydrogen bond occurs, the energy required to break the hydrogen bond would decrease and the activation energy of dielectric relaxation at a constant volume should therefore decrease with increasing temperature. The following consideration shows that it is so. The activation energy at constant volume, E_v^* , and constant pressure, E_p^* are related by

$$E_p^* = E_v^* + (\alpha/\beta)T\Delta V^* \quad (3)$$

where ΔV^* is the volume of activation. Differentiating equation (3) with respect to temperature:

$$(\partial E_p^*/\partial T)_P = (\partial E_v^*/\partial T)_V + (\alpha/\beta)\Delta V^* + (T\Delta V^*/\beta)(\partial \alpha/\partial T)$$

For ice near 255 K, $\Delta V^* = 3 \text{ ml mol}^{-1}$ (ref. 7), $(\partial \alpha/\partial T)_P = 7.39 \times 10^{-7} \text{ K}^{-2}$ (ref. 8), and $(\partial E_p^*/\partial T)_P$ is zero⁹. Therefore, $(\partial E_v^*/\partial T)_V = -9 \text{ J mol}^{-1} \text{ K}^{-1}$.

The simplest consideration of anharmonicity leads one to a negative value of $(\partial \nu_1/\partial T)_V$, for anharmonicity generally reduces the force constant and consequently the frequency of a vibrational mode, when a system is excited to higher energy levels by raising the temperature at a constant volume. It is conceivable that terms higher than the cubic predominate in the potential energy of O-H stretching and thus anharmonicity may play a greater role in determining the temperature dependence of ν_1 . The intermolecular coupling of O-H vibrations at a constant volume is affected by both the lengthening of the hydrogen bond and the anharmonicity of the potential energy. Its magnitude is difficult to assess without the knowledge of the other two effects.

The above results are significant because it is widely believed that the vibrational frequencies depend largely on the volume and because the results may be helpful in a detailed analysis of the vibrational spectrum of both the crystalline^{10,11} and vitreous ices² from the point of view of solid state spectroscopy. They are of further value, because the corresponding measurement of the IR spectra of hexagonal ice is difficult.

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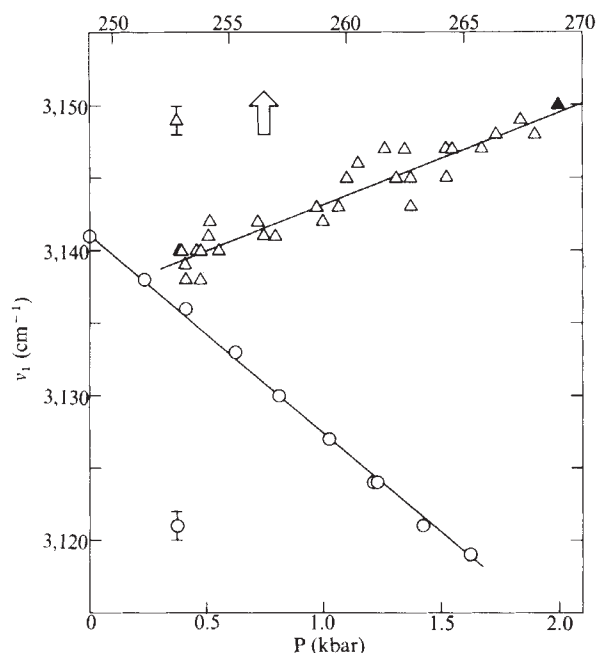
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Fig. 2 The frequency of the in-phase O-H stretching vibration, of hexagonal ice plotted against temperature at 1 bar at the top and against pressure at 255 ± 0.3 K at the bottom. Filled triangle is from an isotropic spectrum from ref. 12.



Collection of atomic mercury by electrostatic precipitators

ELECTROSTATIC precipitators are widely used to remove dust particles from gases and the mechanism of this is well understood. One major application is in the cleaning of a sulphur dioxide produced in smelting of metalliferous sulphide ores before its conversion to sulphuric acid and the precipitated material is often found to contain mercury. We have shown that the concentration of mercury is below the saturation level and therefore present as atoms so the accepted mechanism for solid particle collection is not involved in mercury removal. We have investigated the phenomenon in the laboratory using air as the carrier gas at room temperature, and by large scale tests carried out in an 800 tonne d^{-1} sulphuric acid plant treating the gases formed by roasting zinc sulphide concentrate. In both types of test a discharge wire and earthed collector tube precipitator configuration was used. In the plant tests the roaster gas contained 9% sulphur dioxide and was saturated with water. In both the laboratory and plant tests the gas streams were unsaturated with respect to elemental mercury vapour.

Flameless atomic absorption spectroscopy was used to measure the difference in the mercury concentration of gas entering and leaving a precipitator. This technique is specific for the detection of discrete gaseous atoms, which suggests that gaseous atomic mercury, and not particles containing mercury, is removed by electrostatic precipitators.

In our tests, mercury was only collected during or after generation of a corona discharge (Fig. 1). When nitrogen was used as a carrier gas, mercury was not collected because nitrogen will not sustain a corona discharge¹.

We postulate that in the corona discharge, high energy ions bombard the electrode surfaces and disrupt a passivating layer, such as an oxide, hence producing a more reactive surface. When mercury atoms diffuse to the electrode surface they are adsorbed on the active sites.

Mercury was collected by both electrodes, irrespective of discharge wire polarity, and by a variety of electrode materials including lead, copper, tin, aluminium and stainless steel.

Following voltage application, mercury collection efficiency increased gradually to a steady-state value which was independent of the electrode material. The time taken to achieve the steady-state condition with respect to the mercury collection varied with electrode material (Fig. 1), reflecting the time required to clean electrode surfaces of different chemical composition. The extent of cleaning, and hence the steady-state mercury collection efficiency, depended on the voltage. Mercury

Fig. 1 Time dependence of elemental mercury collection in an electrostatic precipitator. *a*, Voltage on 880 kV m^{-1} ; *b*, voltage off, air velocity, 0.084 m s^{-1} . $\times$, Copper tube; $\circ$, aluminium tube both 35 mm diameter.

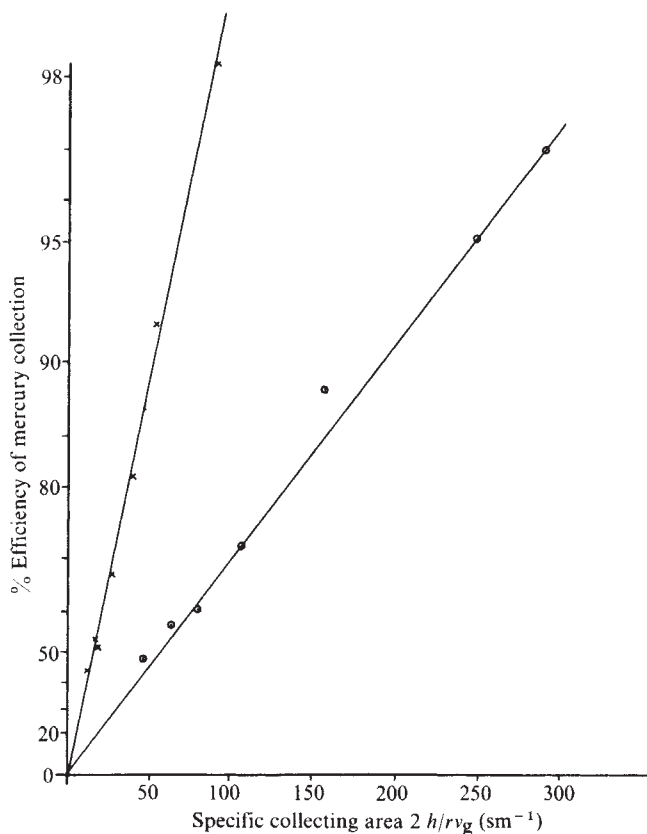
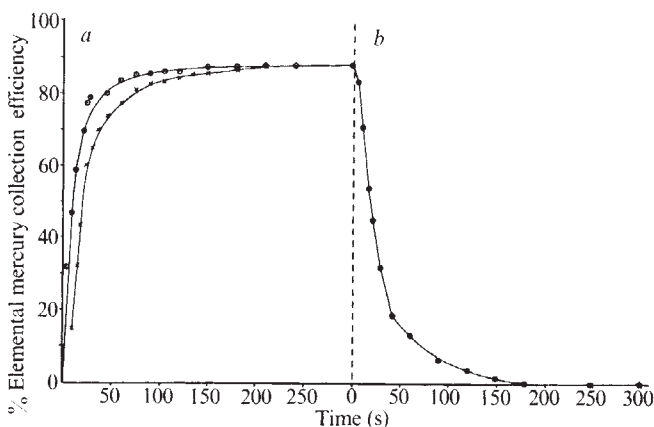


Fig. 2 Variation of mercury collection efficiency with precipitator specific collecting area at constant electrical field strength. $\times$, Tube radius 8 mm, electrical field strength 800 kV m^{-1} ; $\circ$, tube radius 23 mm, electrical field strength 520 kV m^{-1} .

collection continued after the voltage had been switched off (Fig. 1) the efficiency of collection decreasing continuously to zero as the electrode surfaces passivated. The rate at which the efficiency of mercury collection decreased was substantially slower than the decay of the precipitator voltage. If the collection of mercury ions was involved, mercury collection efficiency would have decreased discontinuously when the voltage was switched off, because ions would only be formed when the voltage was applied.

For a precipitator operating with a negative discharge wire and an earthed collector tube, the efficiency of atomic mercury collection can be described by equation (1), which is derived from the Deutsch equation²

$$E = 100[1 - \exp(-k2h/rv_g)] \quad (1)$$

where E is the percentage efficiency of mercury collection, r and h are the collector electrode tube radius and length, respectively, v_g is the axial gas velocity, and $(2h/rv_g)$ is the specific collecting area of the precipitator (electrode surface area/gas flow rate), and k is a complex function incorporating the rate of transport of mercury atoms to the electrode surfaces and their probability of collection. k varies with tube radius and voltage according to equation (2)

$$k = AV/2r \quad (2)$$

where A is a constant for a given tube radius and V is the applied voltage.

Equation (1) was verified experimentally (Fig. 2) over a wide range of conditions ($r = 8.0\text{--}45 \text{ mm}$ for laboratory and $r = 75 \text{ mm}$ for plant tests). A maximum mercury collection efficiency in excess of 99% was achieved. The efficiency of

mercury collection was independent of the mercury concentration in gas entering the precipitator for concentrations in the range $0.1\text{--}8\text{ mg m}^{-3}$.

At very high discharge voltages equation (1) may over-estimate mercury collection efficiency because the gaseous ions bombarding the electrode surfaces can become sufficiently energetic to desorb collected mercury. In the absence of a corona discharge, no mercury was released when mercury-free air was passed through a precipitator which had previously been used to collect mercury, proving that mercury was bound to the electrode surfaces. However, when a discharge voltage greater than the threshold value for which equation (1) is valid was applied, mercury was desorbed into the mercury-free air. The threshold voltage for mercury desorption was independent of electrode material.

For precipitators operating at a constant voltage below the threshold voltage for mercury desorption, A and hence k (equations (1) and (2)) increases with decreasing tube radius for $r < 35\text{ mm}$, but seems to be independent of tube radius when $r > 35\text{ mm}$. Because k increases as r decreases below 35 mm the electrode area required for a given application can be decreased by designing a precipitator with a very small electrode separation.

We suggest that electrostatic precipitators with smaller than normal electrode separations would be capable of removing mercury and perhaps other volatile environmentally undesirable elements, such as selenium and tellurium, which are present as vapour in the gas streams produced during the processing of ores and the burning of certain types of coal.

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Molecular morphology of polyethylene determined by NMR

SOME of the earliest applications of nuclear magnetic resonance (NMR) concerned molecular structural determinations in the solid state via proton NMR second moments, a sensitive function of the number, types and relative positions of the constituent nuclei. This classical technique has been extended to polymeric solids, but it has not previously been exploited to address the problem of molecular morphology in polymer crystals. We describe here a framework for the interpretation of such measurements in cocrystals of polyethylene (PEH) and perdeuteropolyethylene (PED) and present preliminary experimental data. The potential of this technique for distinguishing between adjacent re-entry and random re-entry molecular morphologies on a very local scale complements the ability of small angle neutron scattering (SANS) to ascertain the larger scale configuration of the polymer chain.

The bulk morphology in semicrystalline polymers is well understood. It can be described superficially as a dispersion of crystalline lamellae (with dimensions of the order of 10^{-6} m on the edges and 10^{-8} m in thickness) in an amorphous matrix. By contrast, only a limited number of features of the molecular morphology within the lamellae have been unequivocally defined^{1,2}, and these are all functions inherent in the primary

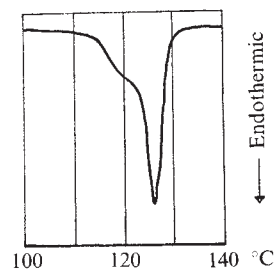


Fig. 1 The melt endotherm for an etched, solution-crystallised polyethylene co-crystal with $x^0 = 0.53$; heating rate 5°C per min ; DuPont 990 thermal analyser. The PED aggregates in the co-crystal. The low temperature shoulder, approximately 15% to 25% of the total endotherm, is attributed to the PED phase resulting in a range for the mole fraction of PEH in the co-crystal, $x = 0.64$ to 0.72 . (Note, our observed melting point for quenched, unetched PEH is 136°C .)

structure of the polymer: (1) the extended, low energy chain conformation; (2) the lateral juxtaposition of the chains; and (3) the orientation of the rectilinear chain axis normal to the lamella surface.

The latter feature has prompted controversial hypotheses to explicate the mode of re-entry of a polymer chain into the lamella surface, a 001 lattice plane. The adjacent re-entry (AR) model posits a series of regular folds (180° turns) that dictate re-entry of the chain into the lamella at an adjacent, nearest-neighbour site in the 001 plane³. The random re-entry (RR) or 'switchboard' model imposes no such external constraints on the trajectory of the chain. The RR model stipulates that, on emerging from the lamella, the chain either (1) re-enters the same lamella at a random site, which could be far removed from the point of emergence (subject to the stereochemical and linear dimensional constraints of the polymer chains) or, (2) permeates the interlamellae amorphous matrix and enters a neighbouring lamella^{4,5}.

Recent SANS investigations of co-crystallised mixtures of PED and PEH have rekindled this controversy⁶ and have been used as supporting evidence for both the AR^{7,8} and the RR^{9,10} models, depending on the method of sample preparation. By way of circumventing this interpretive dilemma we pursue a different experimental approach to the problem—NMR, a sensitive technique for probing the local environment of a molecule. Proton NMR second moment measurements¹¹, typically used to investigate the bulk morphology¹² and relaxation processes¹³ in semicrystalline polymers, provide an entree to the problem.

For protons in a polycrystalline sample, the second moment is¹⁴

$$M_2 = \frac{3}{4}\gamma^2\hbar^2I(I+1)\frac{1}{N}\sum_{j,k}(r_{jk})^{-6} \equiv M_2^a + M_2^s \quad (1)$$

where γ is the proton gyromagnetic ratio, its spin $I = 1/2$, N is the number of nuclei in the sum and r_{jk} is the distance between nuclei j and k . In equation (1) we decompose M_2 into its intramolecular part M_2^a and its intermolecular part M_2^s . The former can be calculated from the molecular structure and conformation, the latter from the crystal structure. (In the polymer crystal, we will consider the analogous quantities, the intra- and interstem contributions to M_2 , where the term 'stem' refers to a single traverse of the chain through the lamella.) Non-resonant nuclei I' can also contribute to M_2 but with an additional reduction factor (relative to protons)¹⁵:

$$M_2^s = \frac{4I'(I'+1)\gamma'^2}{9I(I+1)\gamma^2} M_2 \quad (2)$$

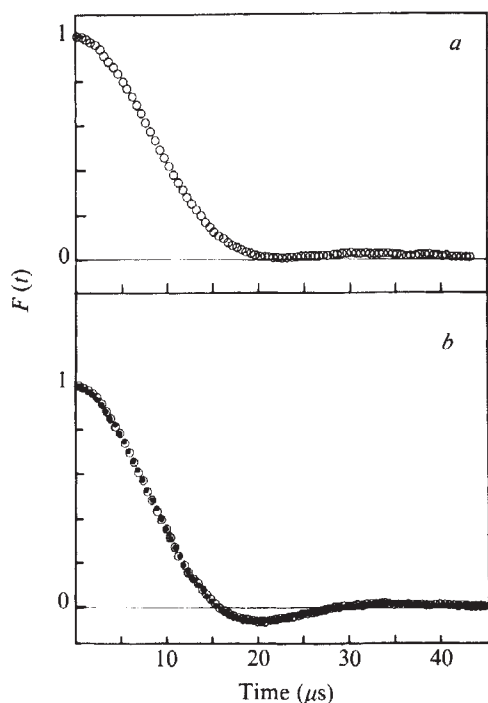
where I' and γ' are characteristic of the non-resonant nucleus.

For deuterium ($I' = 1$), this reduction amounts to ≈ 0.02 of that anticipated from protons at comparable r_{jk} .

Samples of co-crystallised PEH and PED (molecular weights 2.0×10^5 and 2.8×10^5 , respectively) were prepared in two ways: in Method I, quenched polyethylene, 1% solutions of mixtures of PEH and PED ($x = 1$ to 0.28; x = mole fraction of PEH) refluxed in orthodichlorobenzene for 30 min under N_2 were quenched (precipitated) in an excess of methanol, washed with methanol and dried under vacuum at 65 °C (48 h); in Method II, solution-crystallised polyethylene, a 0.1% solution of PEH and PED ($x = 0.53$) in refluxing orthodichlorobenzene was rapidly cooled (~ 2 min) to 90 °C and allowed to stand (48 h). The resulting crystals were collected at 90 °C, extracted with acetone in a Soxhlet (24 h) and dried as in Method I. In order to exclude contributions to M_2 originating from the amorphous fraction of the polymer (see Fig. 2) all samples were subjected to a fuming nitric acid etching procedure (80 °C for 96 h); the highly crystalline debris was washed in H_2O , extracted with acetone (24 h) and dried under vacuum at 65 °C (24 h).

For all co-crystals calibrated NMR intensity measurements show that $\bar{x}$, the mean mole fraction of PEH in the co-crystal, is identical to that initially present in the solution blend, x_0 , that is, PEH (PED) is not partitioned preferentially into the amorphous phase and lost during etching. Aggregation of PEH (PED) into distinctly separate phases can be checked by examining the melting endotherms of the co-crystals⁹. Single endotherms are observed for quenched, etched co-crystals. A shoulder on the low temperature side of the endotherm is indicative of PED aggregation in the co-crystal. In the case of solution-crystallised mixtures of PEH and PED, the aggregation of the PED is pronounced (Fig. 1). In the resulting co-crystal, the local mole fraction of PED, x , is obviously not the same as x_0 . We have graphically decomposed this endotherm into two components, the melting of a PEH/PED co-crystal at 126 °C and the melting of aggregated PED at 122 °C. We estimate that the PED aggregate accounts for 15% to 25% of the entire endotherm

Fig. 2 The proton FID signal in polyethylene following a $90^\circ_0 - \tau - 90^\circ_{90}$ pulse sequence. The signals were obtained with a Bruker pulse spectrometer at 30 MHz; 90° pulse length = 1.5 μ s; $\tau = 10$ μ s; $T = 20$ °C. Typically 256 FID signals were averaged using a Biomation 802 digitiser and a Nicolet 1072 signal averager. $\circ$, Experimental FID. *a*, Quenched PEH. *b*, Quenched and etched PEH: $\bullet$, computed FID



and, assuming the same ΔH for PEH and PED, x ranges from 0.64 to 0.72 for this co-crystal. From the melting points of these co-crystals we estimate lamellae thicknesses of 150 Å and 180 Å for solution quenched and solution-crystallised samples, respectively¹⁶.

The NMR absorption line shape and the free induction decay (FID), $F(t)$, are related by a Fourier transform. Even moments M_n of the resonance line shape are related to the coefficients of an even power series expansion of $F(t)$ as¹⁵

$$F(t) = \sum_{\substack{n \\ \text{even}}} \frac{M_n t^n}{n!} \quad (3)$$

A fit of the observed FID to this power series was used to compute the second moments. Figure 2 contrasts the FIDs of unetched and etched PEH and illustrates a computed fit to equation (3). The value for M_2 obtained from quenched PEH at 20 °C is 26.3 ± 0.5 G², and lowering the temperature to -150° produced no observable changes in M_2 . As noted previously, when the amorphous fraction is etched from PEH, the rigid lattice limit obtains at 20 °C (refs 17, 18). The observed M_2 is, however, larger than that previously reported for etched PEH after correcting for thermal expansion of crystal lattice (24.3 ± 0.5 G²).¹⁷ While the reproducibility of M_2 is excellent, the absolute magnitude computed from the FID is limited by the accuracy of determining the position of the dipolar echo ($t = 0$) following the $90^\circ_0 - \tau - 90^\circ_{90}$ pulse sequence. Once a procedure for determining $t = 0$ is established, relative values of the second moment, $R = M_2/26.3$, can be confidently compared.

Isotopic dilution experiments can be used to separate R into the intra- and interstem contribution to M_2 , R^a and R^i , respectively. The interstem contribution is scaled by the conditional probability $P(H|H)$ that a given nearest-neighbour (site) of a protonated species is also protonated. In order to extract the details of the molecular morphology in the lamellae we calculate S , the interstem contribution to M_2 per site in the crystal relative to that for pure PEH, for the following models of chain re-entry: (1) AR, adjacent reentry with no restrictions on the direction of chain propagation in the 001 plane of the lamella; (2) AR-110, adjacent re-entry with the chain restricted to enter the lattice along the 110 planes; (3), AR-100, adjacent re-entry restricted to the 100 planes; (4) RR, random re-entry of the chain.

In the 001 plane of the crystal each lattice site has six adjacent sites, two along the 100 plane and two each along the 110 and $\bar{1}\bar{1}0$ planes. The absolute computed interactions along the crystal planes, $^{100}M'_2$ and $^{110}M'_2$ depend on the lattice cell constants. However, over a range of cell dimensions the relative interactions $^{ijk}R' = ^{ijk}M'_2/M_2$ remain invariant: $^{100}R' = 0.13$ and $^{110}R' = ^{\bar{1}\bar{1}0}R' = 0.186$.

For the AR model, around each PEH site at least two adjacent sites must also contain PEH chains ($P(H|H) = 1$, ignoring chain ends). We assume that chains with just two self-adjacent sites occur with a probability of 1/3. For multiple self-adjacent re-entries (3, 4, 5, or 6) about a given site we assign a probability of 1/6. The relative contribution S is obtained from the probability weighted sum of $^{ijk}R'$ arising from the different possible configurations about a given site. For the above parameters, one obtains

$$S(\text{AR}) = \frac{22}{36} + \frac{14}{35}x \quad (4)$$

In the AR-110 model, $^{110}P(H|H) = 1$ and $^{100}P(H|H) = x$. Thus

$$\begin{aligned} S(\text{AR-110}) &= 2^{110}P(H|H)^{110}R' \\ &\quad + 2[^{110}P(H|H)^{1\bar{1}0}R' + ^{100}P(H|H)^{100}R'] \\ &= 0.37 + 0.63x \end{aligned} \quad (5)$$

Similarly, $S(\text{AR-100})$ is given by

$$S(\text{AR-100}) = 0.26 + 0.74x \quad (6)$$

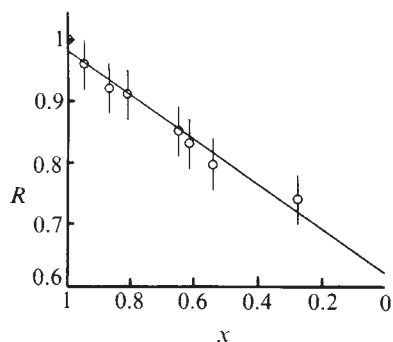


Fig. 3 The relative second moment R plotted against x for solution-quenched and etched co-crystals of PEH and PED; $x = \bar{x} = x_0$ for all samples. The solid line is a linear regression fit to the data with a correlation coefficient of 0.86; $R(x=0) = 0.62 \pm 0.04$; $R(x=1) = 0.98 \pm 0.04$.

Finally, in the RR model, where $P(H|H) = x$ for all sites we obtain

$$S(\text{RR}) = x \quad (7)$$

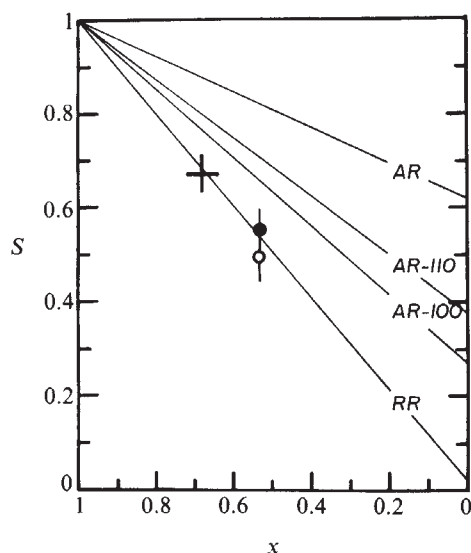
In each of these models the contribution that PED chains in adjacent sites makes to M_2' can be obtained with equations (4)–(7) by replacing x with $(1-x)$ and multiplying the result by 0.02.

To determine which model corresponds to the experimental data, we consider the behaviour of the relative second moment with the isotopic content of the co-crystals. R should be a linear function of x for all the models if the co-crystals are homogeneous. The intercept consists of the intrastem contribution plus the residual interstem contributions characteristic of the molecular morphology. Our procedure is to compute R^a from the intercept of R versus x for the quenched PEH/PED co-crystals (Fig. 3) and the expressions for S of equations (4)–(7) according to

$$R^a = [(R - S)/(1 - S)]_{x=0} \quad (8)$$

The values of R^a computed in this fashion are 0.02, 0.40, 0.47, and 0.62 ± 0.04 respectively, for the AR, AR-110, AR-100 and

Fig. 4 The relative intermolecular contribution to M_2 , S , plotted versus x , the mole fraction of PEH in co-crystals of PEH + PED. ○, Quenched, etched co-crystal; $x = x_0 = 0.53$. ●, Quenched, annealed (123 °C; 48 h), etched co-crystal; $x = x_0 = 0.53$. +, Solution-crystallised, etched co-crystal; x ranges from 0.64 to 0.72 (see Fig. 1).



RR models of chain re-entry. These values of R^a can be compared with the values for R^a calculated for plausible structural and lattice parameters. It should be noted that calculated values for R^a are extremely sensitive to the choice of the bond angles and bond lengths used to compute M_2' (ref. 13). The calculated values of R^a range from 0.67 to 0.73. Thus, from the magnitude of the intercept, there is strong evidence for a random molecular dispersion of PEH in PED characterised by the RR molecular morphology in the quenched co-crystals. The observed $R^a = 0.62 \pm 0.04$ is lower than anticipated and probably reflects, as suggested by Olf and Peterlin,¹⁷ a decrease in M_2' caused by the presence of $-\text{COOH}$ and $-\text{NO}_2$ groups near the chain ends at the etched lamella surface. On the other hand, heterogeneity due to phase separation in the co-crystals would, for a given x_0 , yield larger experimental values for R and result in a correspondingly larger apparent R^a .

Now we consider the relative interstem contributions to M_2 . In Fig. 4 the calculated values of S (equations (4)–(7)) are shown plotted against x for the four models of chain re-entry. Also shown are three experimental values for relative interstem second moments derived from $S = (R - R^a)/(1 - R^a)$ with $R^a = 0.64 \pm 0.04$. The experimental values correspond to PEH/PED co-crystals ($\bar{x} = x_0 = 0.53$) that differ in their methods of preparation. Both samples of the solution-quenched co-crystals (one annealed at 123 °C for 48 h before etching) exhibited single, sharp melting endotherms indicative of homogeneous co-crystals characterised by $x = \bar{x}$. According to Fig. 4, within the experimental uncertainty, the annealing treatment does not significantly perturb the RR molecular morphology in the solution-quenched co-crystals. In the solution-crystallised sample, the fact that $\bar{x} = x_0$ merely indicates that the crystallisation was carried out to 100% conversion. Severe compositional heterogeneity can occur in solution-crystallised mixtures of PEH and PED¹⁹. However, the profile of the melting endotherm of this sample (Fig. 1) prompts us to suggest that only two phases are present: one, pure PED crystals ($x = 0$), comprising 15% to 25% of the sample, the other, co-crystals of PED and PEH characterised by $x = 0.68 \pm 0.04$. For this compositional distribution, the RR model again accounts for the observed value of S . (Note that using a value of $R^a > 0.64$ yields smaller values for S .)

In contrasting the observed values of S with the predictions of the model morphologies, the procedure used does presume compositional homogeneity, that is, x must be representative of the distribution of PEH throughout the macroscopic sample of co-crystals. In heterogeneous samples, specification of the distribution of composition is prerequisite to the extraction of molecular details from measurements of intensive parameters such as NMR second moments. (One can, for example, arrive at the observed value of S for the solution-crystallised co-crystal with the AR-110 morphology assuming that the low melting fraction (Fig. 1) has $x = 0.2$ and the remaining fraction is characterised by $x = 0.6$; $\bar{x} = 0.53$ for the entire sample.)

In conclusion, the NMR second moment data provide another perspective on the nature of the molecular morphology in polyethylene. The NMR experiments, unaffected by the overall configuration of the chains, complement the neutron scattering experiments in the sense that they selectively probe the local short-range structure. The NMR reconstruction of the mode of chain re-entry into the lamella appears to be independent of the method of sample preparation. While unequivocal data on solution-crystallised polyethylene requires compositionally homogeneous co-crystals (perhaps accessible through fractional crystallisation), these preliminary NMR results argue against theories of regular chain re-entry. Most significantly, the NMR method should be able to discriminate among less ideal models of molecular morphology, that is, two (three, ...) AR stems followed by a random trajectory, followed by another sequence of AR stems, and so on, until the entire polymer chain is incorporated in the crystal.

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Note added in proof: R. Voelkel and H. Sillescu have independently applied the NMR isotopic dilution method to PEH and observe the molecular morphology to be molecular weight dependent (manuscript in preparation).

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Soil stabilisation by cellulose xanthate

CELLULOSE XANTHATE is unusually effective in stabilising soil against attrition and deformation. At a concentration of about 0.1% in the soil it reduces erosion; at higher concentrations it can be used to bind soils to improve their mechanical strength. During an investigation of other uses for cellulose xanthate, we recently rediscovered its unusual interaction with soils. We describe here its formation and application in the laboratory, quantitatively compare it with several synthetic polymeric soil conditioners and suggest a mechanism for its action. With regard to the effectiveness of the xanthate soil treatment, its economy and the ready availability of agricultural waste cellulose, we hope to encourage further study of this interesting system.

The initial disclosure of soil stabilisation by cellulose xanthate was a series of patents by Meadows¹, followed by two brief reports by another group^{2,3}. As far as can be determined no further use of cellulose xanthate for soil treatment has been made. A number of other materials have been proposed or used for soil stabilisation, for example, strong composite structures can be made from polymers, asphalt or Portland cement⁴, in amounts of the order of 10% of the soil. At lower concentrations, various hydrophilic polymers, usually polyelectrolytes, can be used as conditioners to stabilise aggregates and improve tilth⁵. Widespread use of soil additives is cost limited. Experience in applying cellulose xanthate should determine whether it is significantly cheaper than additives based on petrochemicals or energy intensive sources.

Cellulose xanthate can easily be made from any cellulosic source that contains accessible alpha cellulose: rice or wheat straw, chemically pulped wood or paper products, cotton, and similar materials. Mechanically pulped wood or papers are not generally useful, as the structure resists solution after xantha-

tion. In this work cellulose xanthate solutions were made from used computer cards, a source of nearly pure fibrous cellulose. The cards (~2.5 g each) were soaked for 10 min in 18% (w/w) aqueous NaOH, drained and padded to dampness with paper towels. The damp alkali cellulose was transferred to a wide-mouth jar, and 1.5 ml carbon disulphide was added for each card. After rapid mixing the jar was tightly closed. Several hours later the xanthate formed an orangish sticky mass at the bottom of the jar. Using a mechanical stirrer, the xanthate was diluted to a 2% solution (based on cellulose content), which was of low enough viscosity to facilitate later measured dilutions. The shelf life of aqueous xanthate solutions is short, a few days at most, so experiments were initiated soon after preparation. Cellulose is regenerable from the xanthate solution by acidification, heating, increasing the ionic strength, or merely by waiting. In soil treatment with xanthate, hastening regeneration is generally unnecessary.

Imperial Valley clay (California) was used to test the stabilising effect of cellulose xanthate. After rough grinding to remove lumps, 20 samples of 20 g each were prepared. This clay was found to form a thick paste when 5–6 ml of water was mixed with 20 g clay. Thus, to obtain a series of samples with known cellulose content, appropriate amounts of the 2% stock solution of cellulose xanthate were diluted to about 5 ml and mixed with the clay. By this method, treatment levels of 0.01, 0.025, 0.05, 0.1, 0.25 and 0.5% cellulose (dry weight) were added to the clay. Two control samples were similarly prepared with water only. Partially dried treated clay was stirred to form irregular balls about 1 cm in diameter. After air drying and curing for a minimum of 24 h at room temperature, the aggregates were sized to pass a 2 mesh (12.7 mm) but not an 8 mesh (3.2 mm) screen. The dry weight of each sample of sized aggregates was redetermined.

To estimate relative stability, a sample was placed in a 236-ml bottle with 125 ml of water. After 1 min, the bottle was rotated end over end at 30 r.p.m. The dry weight of fines that passed through a 20 mesh (1.3 mm) screen was determined after agitation periods of 1, 4, and 16 min. Weight losses are given in Table 1.

For comparison, two synthetic polymeric soil conditioners were also tested: polyvinyl alcohol (PVA) and a hydrolysed polyacrylonitrile (HPAN). These conditioners were applied at the same treatment levels as the cellulose xanthate and tested in the same way with the rotating agitator. Results are also shown in Table 1.

Qualitative differences in texture were noted among the various treatments after drying: both the xanthate and HPAN treatments gave distinctly hardened aggregates, whereas the PVA produced more crumbly material. At higher treatment levels the xanthate-treated soil was especially firm when wet, with a rubber-like springiness. The weight loss during agitation also occurred differently among the three treatments. The rotatory test was rather harsh and, as the test progressed, xanthate-treated and to a lesser extent HPAN-treated aggregates tended to become rounded, while the PVA treatment gave irregular aggregates that gradually broke up. Weight loss of the xanthate-treated clay is, in most cases, substantially less than that of the PVA- or HPAN-treated clay (Table 1).

The mechanism of soil stabilisation by cellulose xanthate may be unique in that it seems to depend initially on adsorption as a polyelectrolyte and then later on the regeneration of cellulose. The behaviour of cellulose xanthate as a polyelectrolyte is well known⁶, as is the effectiveness of polyelectrolytes in binding together clay particles⁷. The xanthate as applied is the ionised sodium salt of the polyanion; hence, its initial interaction with soils should be largely by adsorption to the surface of the clay component. This is apparent from the immediate stabilisation of clay by xanthate, noticeable even when still wet; this rapid stabilisation does not occur with sands.

After some time, most of the xanthate is regenerated to cellulose; cellulose fibrils then presumably take the place of adsorbed xanthate to bridge between particles and confer

Table 1 Percentage weight loss of Imperial Valley clay aggregates treated with various amounts of soil stabilisers

Wt % stabiliser added	1 min wash			4 min wash			16 min wash		
	Cell	Additive PVA	HPAN	Cell	Additive PVA	HPAN	Cell	Additive PVA	HPAN
0 (control)	48	(48)	(48)	84	(84)	(84)	100	(100)	(100)
0.01	36	39	47	72	75	82	100	100	100
0.025	25	39	45	56	75	80	94	100	100
0.05	14	40	37	41	72	70	83	100	100
0.1	7	39	39	26	69	74	63	100	100
0.25	3	19	33	10	38	64	26	64	94
0.5	2	10	10	6	19	24	17	41	57

Aggregates and stabilisers were agitated in water for 1, 4 or 16 min after an initial 1 min static soak. Stabilisers used were cellulose xanthate (Cell), polyvinyl alcohol (PVA), and hydrolysed polyacrylonitrile (HPAN). Xanthate was prepared as described in the text. The PVA was Du Pont 'Elvanol' polyvinyl alcohol, grade 72-51, 98.5-100% hydrolysed, MW 54,000. HPAN was obtained from a sample of 'Ortho-Til' (Ortho), designated as 83% HPAN; the sample was identified as sodium polyacrylate.

stability. Evidence for the shift from polyelectrolyte sorption in the xanthate form to binding by regenerated cellulose is afforded by analysis for oxidisable sulphur remaining in the treated samples. The analytical method was based on oxidising divalent sulphur by hydrogen peroxide to form the sulphate⁸, after which a slight excess of barium ion was added, and the decrease in soluble barium determined by atomic absorption⁹. Table 2 shows the oxidisable sulphur thus determined for a kaolin sample initially treated with cellulose xanthate to a cellulose level of 0.5%. The results are also given as weight per cent cellulose xanthate (calculated as cellulose), assuming one xanthate group for each two anhydroglucose monomer units. The initial cellulose content calculated from the sulphur content is 0.8%; the amount over 0.5% may represent sulphur present as excess CS₂. After 700 h, the oxidisable sulphur decreased to 2 mg per 100 g kaolin, representing a xanthate content of 0.01% (calculated as cellulose). As clay stabilisation by xanthate seems permanent (barring microbial action), regenerated cellulose must be the effective binding agent in the long run.

Microbial action may limit some applications. The lifetime and fate of the regenerated cellulose in practical applications are unknown, but it appears to last for at least several months³. In our laboratory, samples have been kept in water for more than six months with no marked change. Appropriate antimicrobial agents may be useful for producing a more permanent treatment.

For some applications of cellulose xanthate, its relatively short shelf life in the presence of water may be objectionable. We have found that drying at room temperature and refrigeration are effective in retarding regeneration to cellulose, although these expedients delay rather than prevent regeneration. The reason for this lies in the numerous side reactions attending xanthate formation, two of the more important being



and



The trithiocarbonate is largely responsible for the orange colour of prepared xanthate; it is a stable salt, as is the carbonate and to a lesser extent the sulphide. These reactions are rather slow¹⁰, evidently slower than xanthate formation, which is complete within a few hours at room temperature. Later, however, the reaction tends to be driven toward the formation of thio-carbonate, or at least to the sulphide/carbonate stage if CS₂ is deficient, with the cellulose xanthate decomposing to supply the necessary NaOH and CS₂, along with cellulose. Reducing the temperature slows regeneration, as does removal of water, since ionic reactions are involved. Meadows¹ found that adding a chemical dehydrating agent (for example, CaO) to the air-dried xanthate permitted stable storage for much longer times. Addi-

tional calcium ion is often beneficial for soil applications and this inexpensive addition should ameliorate the stability problem.

Spraying or sprinkling permits direct application of dilute xanthate solutions to large areas. In test plots of 1-4 m², we produced erosion resistance by applying a 0.4% cellulose solution to a depth of 1 cm, leaving a cellulose content of 0.07% in the soil. From adjacent plots tilted to about 20° angles, the silt runoff from an untreated plot was over 500 times that from a treated plot.

In test plots, treated soil appeared to absorb water more rapidly, indicating an increased permeability. This was verified

Table 2 Oxidisable sulphur content of kaolin treated with cellulose xanthate

Time (h)	Oxidisable sulphur (mg per 100 g kaolin)	% Cellulose xanthate in kaolin calculated as cellulose
0	162	0.82
0.2	149	0.75
1	124	0.63
4	123	0.62
20	83	0.42
44	70	0.35
200	36	0.18
700	2	0.01

The analytical values given for zero time were calculated from analysis of the added xanthate solution.

in the laboratory by measuring the volume absorbed by Imperial silty clay standing under a 1 cm head of water for 16 min. Samples were passed through a hand mill to obtain similar particle size and uniformly packed in 9-cm diameter cans by rapping the perimeter with a small rubber mallet. The surfaces of the untreated and bulk treated clay samples were water wetted to 1 cm depth and dried for 24 h before the test; a third sample was wetted with a dilute xanthate solution to 1 cm depth and similarly dried. Untreated clay absorbed 8 l m⁻² of exposed surface. Clay treated to contain 0.1% cellulose in the top centimetre absorbed 12 l m⁻², and clay treated to contain 0.1% cellulose throughout its bulk absorbed 19 l m⁻².

Soil treated with cellulose xanthate does not appear to affect seed germination and growth, as was observed both from weed growth on the test plots and from germination of wheat seeds in the laboratory. However, if the treated soil is compacted with a roller, a hard surface is produced that seems to inhibit seed germination.

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Sedimentation rates determined by ^{137}Cs dating in a rapidly accreting salt marsh

SEA-LEVEL records indicate that the coast of Louisiana and other parts of the Gulf Coast are rapidly subsiding¹. Louisiana is now losing approximately 16 square miles of land per year, primarily to subsidence²; the rates of subsidence vary with location. Vertical marsh accretion is the process which counteracts subsidence and eustatic sea-level rise and prevents marsh deterioration, but, as in Louisiana's salt marshes, the pattern, rate and variability are sufficiently complicated to defy simple prediction. Conditions of marsh development vary throughout the coast, from the modern and Atchafalaya deltas through the abandoned deltas to the Chenier Plain³. In recent years, much of the coastal area such as Barataria Basin has been deprived of river-borne sediment through natural stream diversion and the construction of water-control embankments. In addition, dredging from petroleum operations has altered water flow and sedimentation patterns. The survival and productivity of Gulf Coast marshes depend on the influx and accumulation of sediment that offsets the effect of subsidence and maintains the marsh surface within the tidal range. To predict long-range trends in marsh stability, accurate measurements are needed of both subsidence and sedimentation rates. Information on subsidence is available from tide gauge measurements but no measurements have been made of sedimentation rates in marshland developed on Recent Mississippi alluvium. ^{137}Cs , a fallout product of nuclear testing, has become a useful tool for dating recent sedimentary sequence in lakes⁴⁻⁸. We report here its use in the measurement of sedimentation rates in a Louisiana coastal marsh, the first report of such use in coastal marshes.

Cores were taken from a streamside and inland *Spartina alterniflora* salt marsh and from an adjoining shallow water lake in Barataria Basin in Louisiana (29°13' N, 90°7' W). From the streamside location two sediment cores were taken 7 m inland and 5 m apart parallel to a natural stream. Another set of two cores was taken 45 m inland and 5 m apart parallel to the same stream. A third set of two cores was taken 350 m apart in adjacent Airplane Lake, a shallow 19-hectare lake. Cores were obtained by twisting a thin-walled aluminium cylinder of 15 cm diameter and 53 cm depth into the marsh soil or lake bottom. Compaction was minimal using these relatively large-diameter thin-walled cylinders. The sediment was sectioned into 3-cm increments, dried and ground, and each sample well mixed.

^{137}Cs activity in each section was determined by γ counting of the oven-dried sample using a lithium-drifted germanium detector and multichannel analyser⁹. Organic carbon was determined by dry combustion. Organic matter was estimated by multiplying carbon content by 1.724 (ref. 10). Density was determined from oven-dry sediment in the known volume of each section.

Sedimentation rates were calculated from the peak ^{137}Cs concentration found in the marsh and lake profile which corresponded to 1963, the year of peak ^{137}Cs fallout, and 1954, the first year of significant ^{137}Cs fallout^{6,7}. There should be little mobility of ^{137}Cs in sediment, as it is rapidly adsorbed by clay components of sediment and soil^{8,11}. Also, the sediment captured by the dense marsh grass is unlikely to be reworked.

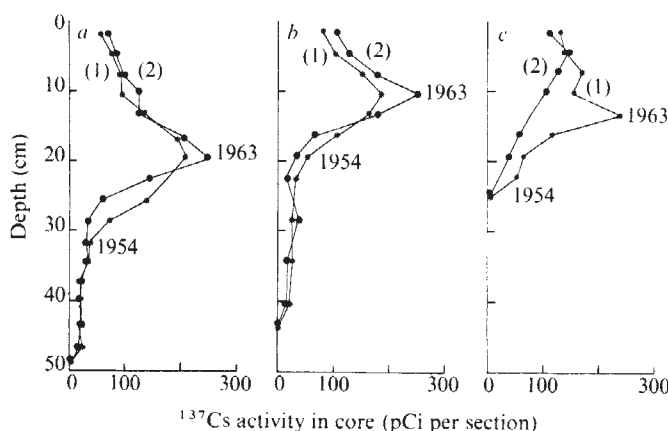
^{137}Cs profile distributions demonstrate rapid vertical marsh accretion (Fig. 1). The two marsh sites nearer the stream were found to be accreting at a rate of 1.35 cm yr⁻¹. The inland marsh was accreting at a slower rate (0.75 cm yr⁻¹). The inland marsh is beginning to deteriorate into small open water areas, presumably because its vertical accretion rate is not able to compensate for subsidence and eustatic sea-level rise. A tail of ^{137}Cs activity extended below the 1954 marker in the marsh profile but not in the lake profiles. This tail corresponds to the rooting depth of *S. alterniflora* and is probably the result of plant absorption of ^{137}Cs (ref. 10). ^{137}Cs activity was expressed on a volume (pCi per 3-cm section) basis.

The distribution of ^{137}Cs in Airplane Lake cores implies accretion rates of 1.1 cm yr⁻¹. A greater amount of sediment mixing occurred in the lake than in the marsh. One of the lake sites did not have a distinct ^{137}Cs peak. However, the 1954 marker horizon for both cores extended to the same depth. A ^{137}Cs tail was not found in the lake cores, apparently because of the absence of plant roots.

The accretion rates reported here are greater than those found in Atlantic coast salt marshes. Richard¹³ recorded accretion rates of 0.34 cm yr⁻¹ in a Long Island salt marsh using brick dust as a marker layer. Armentano and Woodwell¹⁴, using ^{210}Pb , measured accretion rates of 0.47-0.63 cm yr⁻¹ also in a Long Island marsh. The rapid accretion rates obtained by ^{137}Cs dating for this Louisiana salt marsh approximately equal reported subsidence rates of 1.29 and 1.12 cm yr⁻¹ estimated by tide gauge measurements from 1959 to 1971 in this coastal area at Bayou Rigaud (29°16' N, 89°58' W) and Eugene Island (29°22' N, 91°23' W), respectively¹. Even though there is a net land loss along the coast, vertical accretion is apparently compensating for a portion of the subsidence.

Density of the marsh soils ranged between 0.30 g cm⁻³ and 0.10 g cm⁻³, depending on depth and location (Fig. 2). Density of the lake sediment ranged from 0.45 g cm⁻³ at the surface to approximately 0.65 g cm⁻³ at 50 cm. Both mineral and organic matter contribute to the density values. From density and

Fig. 1 Measured ^{137}Cs profiles at each location. a. Streamside 1 and 2; b, inland 1 and 2; c, Airplane Lake 1 and 2.



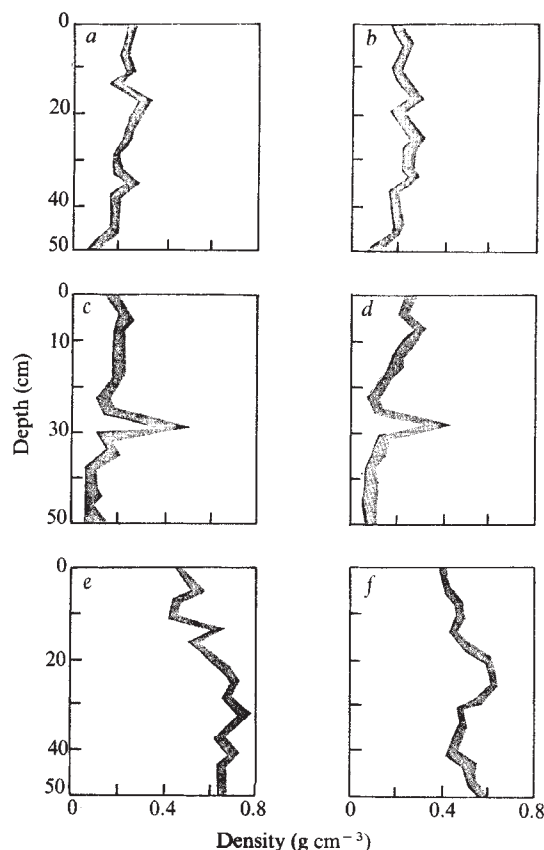


Fig. 2 Distribution of mineral and organic material at each location. *a*, Streamside 1; *b*, streamside 2; *c*, inland 1; *d*, inland 2; *e*, Airplane Lake 1; *f*, Airplane lake 2. Light shading, mineral; dark shading, organic.

carbon content of the cores, it is shown that the area vertically accretes through organic detritus accumulation and sediment input. The lake is receiving a larger amount of mineral sediment than the adjacent marsh.

The entrapment and stabilisation of suspended inorganic sediment on the marsh surface by vegetation is an important process in helping to maintain elevation with respect to sea level. The incoming sediment also supplies nutrients for plants which in turn enhance further sediment entrapment and stabilisation. The increased primary production contributes to the organic pool of these peaty marsh soils. Maintenance of a viable marsh is thus affected through aggradational processes of plant growth, organic detritus accumulation and inorganic deposition.

The ^{137}Cs dating technique thus seems to be useful in determining sedimentation rates over the past quarter-century in salt marshes that are rapidly accreting. Sediment deposited on the marsh surface is stabilised by the dense vegetation and, based on the slope of the ^{137}Cs profiles, there is little reworking and no pronounced biogenic disturbance of the deposited sediment.

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Seismological and geological aspects of the Mantaro landslide in Peru

ON the evening of April 25 1974 one of the largest landslides known took place in the Mayunmarca valley (12.6°S, 74.6°W), adjacent to the Mantaro River, in the Peruvian Andes (Fig. 1). A total of 1,000 to 1,300 × 10⁶ m³ of soil and rock was displaced in successive slides, which occurred within a few minutes of each other. Part of the slide material reached the Mantaro canyon, where a gigantic natural dam was formed. The dam was 3.8 km long extending along the axis of the canyon, 2.5 km wide across the river (Fig. 2), 170 m high from the river bed to the lowest point on the crest, and had a volume of 800 × 10⁶ m³. At the time of the slide, the Mantaro River was discharging nearly 150 m³ s⁻¹, and a reservoir was quickly impounded upstream from the dam. At its largest, the reservoir was approximately 32 km long and contained over 670 × 10⁶ m³ of water. After 42 days, the reservoir overtopped the natural dam. The high potential of flood damage was minimised by construction of a controlled spillway across the dam.

As a direct result of the slide, the village of Mayunmarca and several farms dispersed over 9.6 km² were destroyed. Approximately 400 people lost their lives, but about 200 more would have been killed had they not been evacuated: evacuation had been recommended in November 1973 following a geological study in which the landslide was forecast¹. Large property losses, which included a section of the main road, buildings, and agricultural fields, severely affected the weak economy of this remote region. Large sections of the main road which run parallel to the Mantaro River, were flooded by the reservoir, making the road no longer serviceable.

The Mayunmarca valley is an old glacial valley with a dendritic drainage system, which, before the slide, was formed by several streams cutting fluvial terraces into deep gullies. These streams converged near Mayunmarca and continued to the Mantaro River by the Cochacay gully (Fig. 1). Underground drainage came from the Pumaranra River and from the Yanacocha and Minascocha lagoons, located at elevations >4,000 m. The hills surrounding the valley reach altitudes of >4,300 m, while the bed of the Mantaro River is just under 2,500 m in elevation. These hills are mainly formed by Permian sandstones and shales lying on Palaeozoic schist and phyllites, which are the impermeable basement rocks of the Mayunmarca valley. The sandstones and shales are generally overlain by glacial deposits or by unconsolidated, weathered, and permeable Quaternary alluvium over 100 m thick (Fig. 2).

During the geological study that warned of a possible landslide¹, an abnormally large amount of water in the unconsolidated sediments under the Mayunmarca terrace and signs of recent small slides in the flanks of the surrounding hills were noticed. After the landslide, a geological inspection of the area²⁻⁴ revealed water saturation of the unconsolidated sediments and soils, suggesting loss of cohesion of the upper unconsolidated layers resulting from a rise in the water table and an increase in underground flow. This condition aided in triggering the slide and was helped by the steep gradient of the Mayunmarca valley.

The landslide area has had very low seismic activity in the past 70 yr. Only two seismic events were located in this area, both

being of intermediate depth of focus ($h \sim 100$ km). Local and regional seismic activity during the few days before the slide, as recorded at Huancayo (HUA, $\Delta = 82$ km, $AZ = 327^\circ$) and Ñaña (NNA, $\Delta = 240$ km, $AZ = 284^\circ$), consisted of four small earthquakes that occurred >100 km from HUA, and of a magnitude $m_b = 5.8$ earthquake that occurred approximately 660 km southeast of Mayunmarca and about 17 h before the landslide⁵. Earth tremors were reported near the Mayunmarca valley between 03:00 and 04:00 hours and around 21:00 hours

LT on 25 April. The earlier tremor could correspond to the $m_b = 5.8$ regional event, which occurred 660 km away from Mayunmarca, and the later tremor, to the landslide itself. These possibilities suggest that the landslide was not caused by a seismic event. This hypothesis is supported by the appearance of the short-period seismograms from the landslide and recorded at HUA, NNA, and Arequipa (ARE, $\Delta = 581$ km, $AZ = 138^\circ$), which are similar to the records obtained at Pasadena, California, from slumps originating during petroleum drilling opera-

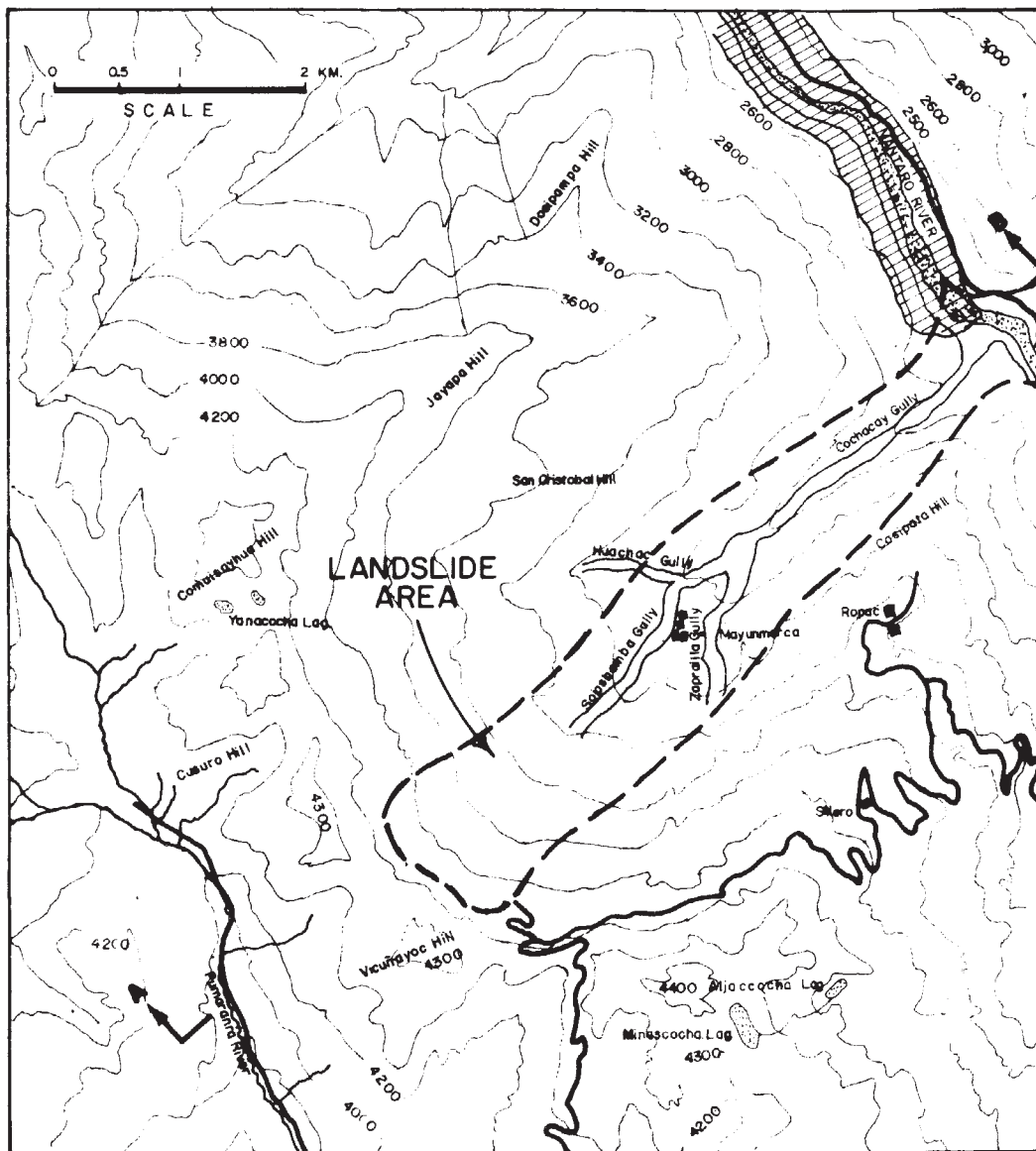


Fig. 1 Location map of the Mayunmarca Valley showing the principal surrounding topographical features (in m) and the area affected by the 25 April 1974, landslide. A-B location of cross section shown in Fig. 2.

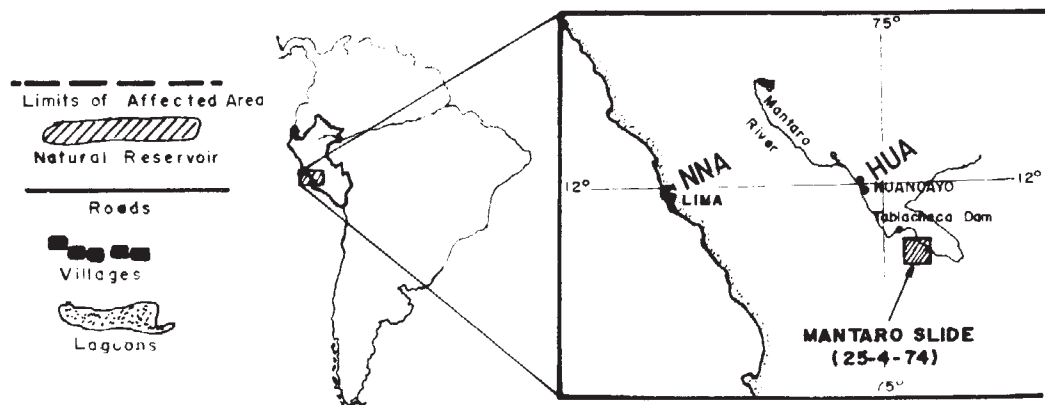
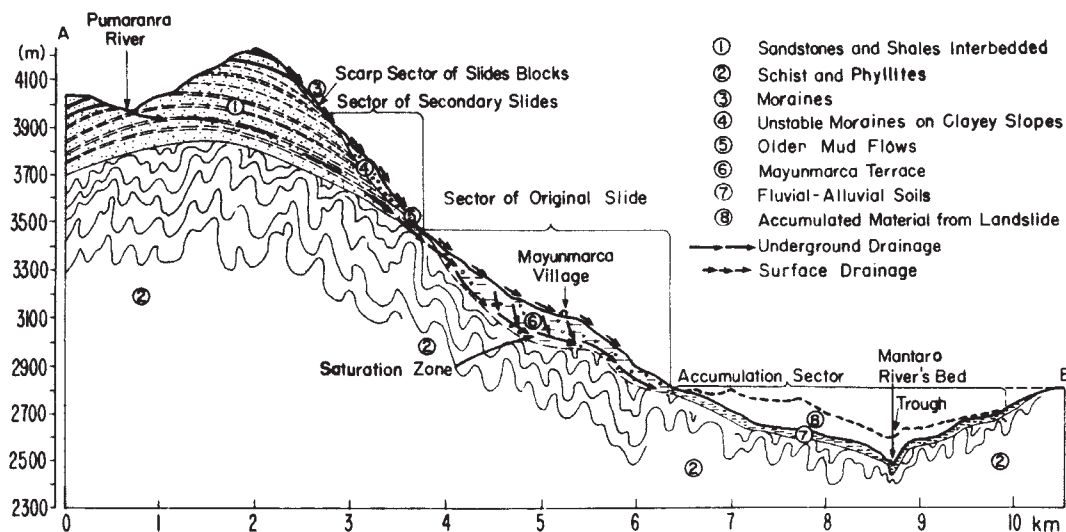


Fig. 2 Section A-B of the Mayunmarca Valley (see Fig. 1) showing major geological and topographical features related with the landslide of 25 April 1974. Vertical scale = $2.5 \times$ horizontal scale.



tions near Terminal Islands⁷. However, the possibility has not been eliminated that a small earthquake, whose magnitude was so small that it was not recorded at the local seismic stations, was the triggering source of the landslide. A seismic survey by the Instituto Geofísico del Perú^{5,6} some weeks after the landslide showed the existence of microearthquakes of possible tectonic origin; they had epicentral distances between 2 and 20 km from the recording site, which was located 20 km from the debris dam and at the upstream end of the reservoir and 3 km from the Tablachaca concrete dam (Fig. 1), which serves the Mantaro Hydroelectric Plant. These microearthquakes could have been associated with the presence of the Tablachaca reservoir or the reservoir created by the Mantaro landslide. A special seismic study could be used to determine the origin of this micro-earthquake activity and to determine whether a fault suggested from surface tectonic features and coinciding with the Mayunmarca valley is active or not.

Seismic waves generated by the Mantaro landslide were recorded at several South American observatories as far away as 2,890 km (ref. 8). Short- and long-period seismograms were recorded at HUA, NNA, and ARE. Only long-period signals were recorded at Las Peñas (PNS, $\Delta \sim 900$ km) and at Brasília (BDF, $\Delta = 2,890$ km).

The origin time of the landslide was 0157 22.5 UTC on 26 April 1974 (2057 22.5 LT on 25 April), calculated from P-arrival times on the HUA short-period seismograms and using Herrin's seismological tables⁹. The disturbance recorded on the HUA and NNA short-period seismograms lasted 4 min, while long-period records show higher frequency waves arriving 3 min after the onset.

From the seismic phases originated by the Mantaro landslide (long-period signals shown in Fig. 3), the geological and physiographical constitution of the Mayunmarca valley before the event¹, and the vertical distribution of different types of debris in the natural dam², we infer that the landslide was a complex phenomenon composed of different slides which occurred during a period no longer than 4 min but longer than $1\frac{1}{2}$ min. The first slide consisted of unconsolidated soils and sediments underlying the Mayunmarca terrace, nearly $3.5 \times 1.6 \times 0.1$ km (560×10^6 m³). After this original slide, the material in the flanks of the surrounding hills became unstable; therefore, most of the remaining material (around 700×10^6 m³) slid in subsequent secondary slides on the top of the affected area and of the debris belonging to the original slide. Large blocks of rocks on top of the surrounding hills also became unstable and fell; some of this material rolled along the disturbed area and reached the natural dam (7 km away) formed by the previous slides.

A large-amplitude phase arriving 70 s after the onset, which was observed on the HUA and NNA short-period seismograms,

points to the impact of the original slide material on the Mantaro canyon, 3.5 km from the Mayunmarca terrace's middle point. This arrival time suggests an average mass-displacement velocity of 180 km h^{-1} .

The kinetic energy developed during the landslide, using an average slide velocity of 180 km h^{-1} , a material density of 2.7 g cm^{-3} , and a total volume of $1,300 \times 10^6 \text{ m}^3$, was about 4.4×10^{22} erg. Using the same physical dimensions as those of the material involved in the landslide, and the distances shown in Fig. 2, the potential gravitational energy of the Mantaro landslide is of the order of 10^{24} erg.

The energy radiated as seismic energy, E , was calculated from local-station seismograms using the relation¹⁰:

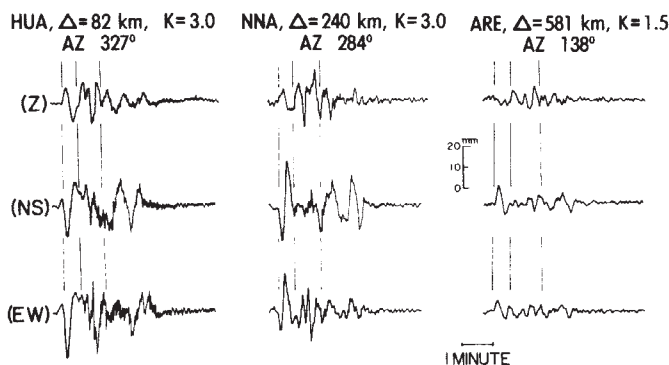
$$\log E = 11.72 + 1.68 M_s \quad (1)$$

The magnitude values, M_s , were calculated from standard equations¹¹.

The values for M_s and E that correspond to the maximum amplitude phases generated by the Mantaro landslide are 3.6 and 6.3×10^{17} erg, respectively, from long-period records, and 3.9 and 18.7×10^{17} erg, respectively, from short-period seismograms.

The M_s value obtained from a BDF long-period seismogram is 4.1, yields an elastic energy of 8.9×10^{17} erg, as computed from the Gutenberg-Richter¹² energy-magnitude relationship. This value is in agreement with the energy values obtained from local-station seismograms.

Fig. 3 Long-period seismograms of the landslide of 25 April 1974 recorded at Huancayo (HUA), Ñaña (NNA), and Arequipa (ARE), showing three seismic phases produced by the landslide. Δ = epicentral distance (km), K , magnification $\times 10^3$, and AZ = azimuth in degrees.



The above results show that seismic energy generated by the 1974 Mantaro landslide was of the order of 10^{18} erg, with a corresponding $M_s = 4.0$. Therefore, the radiated seismic energy is about four orders of magnitude less (0.01%) than the kinetic energy developed during the landslide, which in turn is about two orders of magnitude less (1.0%) than the potential gravitational energy.

The Mantaro landslide is one of the first large landslides to have been widely recorded by several seismic observatories, at local and teleseismic distances. These recordings made calculation of some of the landslide's physical parameters possible. A complete analysis of the seismograms and a detailed interpretation of the landslide mechanism, assessed from such analysis, is in progress.

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Volcanics from the Sierra Leone Rise

THE Sierra Leone Rise, located in the east equatorial Atlantic, forms a discontinuous chain of seamounts as shallow as 2 km extending with a general NE–SW trend from near the Sierra Leone coast of Africa, to the St Paul fracture zone near the Mid-Atlantic Ridge (Fig. 1). The origin of this feature has remained a topic of discussion. Sheridan *et al.*¹ have hypothesised that the Sierra Leone Rise is a volcanic structure formed at the beginning of the opening of the Atlantic in the early Cretaceous period. The twin features of the Sierra Leone and the Ceara Rises are probably of oceanic origin and were created 80 Myr ago or later in their present-day position with respect to Africa and South America². The Atlantic ocean exhibits several similar aseismic structures which appear symmetrically oriented with respect to the mid-oceanic ridge, such as the Walvis–Rio Grande Rise and the Iceland Faeroes–Iceland Greenland Ridges. These structures are volcanic edifices having a composition similar to that found in their associated islands^{3–7}. Deep sea drilling of the Ceara Rise^{8,9} penetrated a basaltic basement of the upper Cretaceous period (Maestrichtian) (Leg 39, Site 354). Similarly, a DSDP hole (Leg 41, Site 366) on the Sierra Leone

Rise, penetrated sediments of the same period, without reaching basement¹⁰. We report here the discovery of alkali-rich volcanics in an area of the Sierra Leone Rise. The sediment overlying the rock fragments is aged ~45 Myr.

During the Romancha cruise of the RV Jean Charcot in December 1977 we studied the south eastern slopes of the Sierra Leone Rise, near DSDP Site 366, with the objective of sampling crustal material (Fig. 1). From a detailed bathymetric survey, using a multichannel narrow beam echo-sounder, it was possible to locate the best existing outcrops for dredging. However, despite this, our dredging operation proved unsuccessful, mainly because of the topography of the area surveyed, which consists of gently rolling sediment-covered hills having slopes of $<10^\circ$.

A sediment core (1.6 m long) was taken in the same area (lat $05^\circ 14.9' N$; long $20^\circ 15.1' W$) as the site of the dredging (Fig. 1). This core was recovered from a depth of 2,720 m near the summit of a seamount, which appeared to be the site of important erosional processes resulting from bottom currents (Fig. 2). This core penetrated a sediment veneer and entered a highly altered basement. The upper levels in the core (0–67 cm) are upper Pleistocene to Holocene in age (NN21–N23) with extensive reworked material as old as middle Eocene, especially from 25 to 67 cm. Despite some disparity between the nanno-fossil and foraminiferal record, only the Oligocene appears absent. From 67 to 110 cm, the ages range from upper to middle

Table 1 Chemical analyses of material collected from a piston core (CH 77–07) from the Sierra Leone Rise

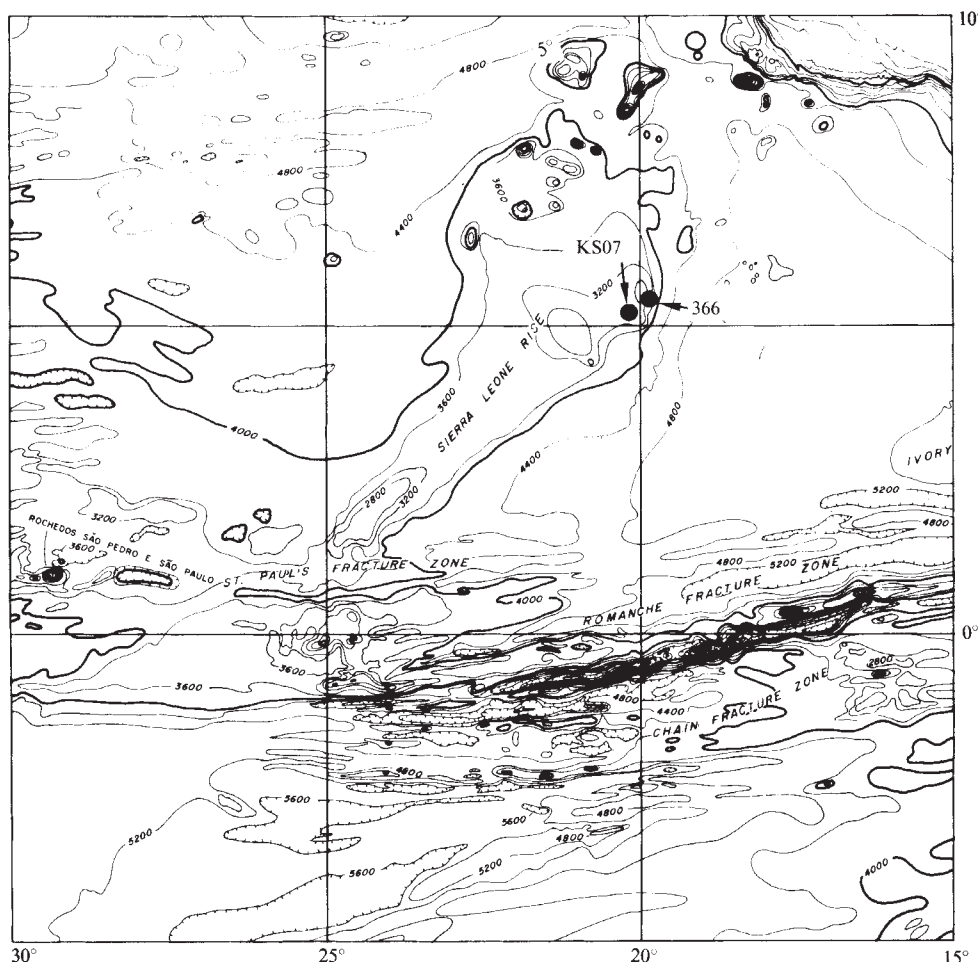
	Clay-like fraction (wt %)	Feldspar in rock fragment	
		Phenocryst	Microphenocryst
SiO ₂	40.95	59.7	64.6
Al ₂ O ₃	11.46	25.7	22.4
Total Fe as FeO	7.73	0.24	0.3
MnO	0.16	—	—
MgO	2.91	—	—
CaO	3.93	7.7	3.1
Na ₂ O	4.03	6.4	9.0
K ₂ O	2.76	0.15	0.6
TiO ₂	1.94	—	—
P ₂ O ₅	2.03	—	—
H ₂ O	15.38	—	—
H ₂ O ⁺	6.12	—	—
Total	99.4	99.89	100.0
Molecular composition	—	Or _{1.9} Ab _{56.7} An _{41.4}	Or _{7.3} Ab _{76.6} An _{16.1}

The small clay-like section matrix made up mainly of smectite and zeolite was analysed by X-ray fluorescence (P. Cambon and J. Etou-bleau). The minerals were determined by energy dispersive analysis of X rays.

Eocene (about 45 Myr BP) with some upper and middle Paleocene (60 Myr BP) foraminifera observed. From 110 to 130 cm, the sediment is a foraminiferal chalk epigenised to apatite. The biogenic elements include fish remains and unidentified casts of foraminifera. Below 130 cm no biogenic elements are observed.

The change in mineralogy corresponds to the stratigraphical limits encountered in the core: from 0 to 67 cm depth in the core, the known carbonate fraction comprising about 15% of bulk material consists mainly of kaolinite with some smectite and quartz. At 67–110 cm the smectites forming the main constituents of this level are associated with well-crystallised zeolite of the heulandite type. This latter material is volcanic in origin. Apatite occurs in the lower section of the core at 110 cm. The contact below 130 cm is made up of a thin (<0.3 cm) iron manganese lamina overlying a green brecciated clay-rich material. This material consists mainly of soft green clay aggregates intermixed with reddish brown abundantly altered and highly vesicular rock fragments, and scattered grains of clino-

Fig. 1 Bathymetric map of the Sierra Leone Rise from Uchupi¹⁴ showing the location of the core CH 77-07 and of the Deep Sea Drilling hole 366¹⁰.



pyroxene and feldspar with sharp crystalline boundaries. The small fraction is formed of well-crystallised smectite, and two types of zeolites, heulandite and phillipsite, are also present in smaller amounts. This small clay-like fraction has a mean composition relatively high in silica (41%), and a high total alkali content ($\text{Na}_2\text{O} = 4\%$, $\text{K}_2\text{O} = 2.8\%$) (Table 1), which is compatible with the mineralogy. The relatively high P_2O_5 content (2.0%) reflects the presence of apatite needles.

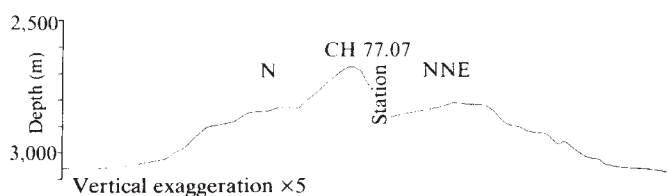
The type of mineral association (pyroxene, feldspar, apatite, smectite and zeolite) is also encountered in the rock fragments (~3 cm diameter) found within the material recovered. These rock fragments consist of early-formed feldspar set in an altered ferruginous matrix containing pyrite, apatite and tiny laths of parallel oriented feldspar indicating a trachytic textural feature. The feldspars in the rocks are tabular with faint twinning and do not show any signs of secondary replacements. The groundmass is reddish brown due to the extensive alteration, with iron oxide material and smectite being the main alteration minerals encountered. Scanning electron microscope observations show that the zeolites developed *in situ* in geode-like structures. Because of the extended degree of alteration in the groundmass, the chemical analyses of the latter did not give any indication of the nature of the rock. However, microprobe (EDAX) analyses of the unaltered feldspar suggest the alkaline character of the samples.

The composition of the feldspar is indicated in Table 1 and shown on a ternary diagram (or-ab-an) in Fig. 3. The potassic and the sodic ($\text{or}_{1.9} \text{ab}_{56.7} \text{an}_{41.4}$ — $\text{or}_{7.3} \text{ab}_{76.6} \text{an}_{16.1}$) nature of the feldspathic phases differs considerably from that found on the accreting plate boundary of the Mid-Atlantic Ridge (Fig. 3). The molecular percentage of orthoclase in the feldspar from the MAR basalts is insignificant^{11,12} when compared with that of the

Sierra Leone Rise. However, there is a similarity in the composition of the Sierra Leone Rise (Fig. 3) feldspar to the alkaline volcanic series from oceanic islands (such as Hawaii, Saint Helena and Gough islands) (Fig. 3). In addition, the mineral composition and the textural features of the rocks from the Sierra Leone Rise suggest the trachyandesite type of volcanism encountered on the Walvis Ridge⁴. The feldspar analysed from the Walvis Ridge alkali basalt (sample WD 3-4) is also of a potassic nature ($\text{or}_{40} \text{ab}_{45.9} \text{an}_{50.1}$ — $\text{or}_{2.4} \text{ab}_{28.6} \text{an}_{69.0}$) like that from the Sierra Leone Rise (Fig. 3).

In conclusion, both the study of the clay fraction and the rock fragments demonstrate that we have sampled an extremely weathered volcanic outcrop near the top of one of the seamounts of the Sierra Leone Rise. This outcrop is covered by a thin intensely reworked carbonate ooze including Eocene to Palaeocene fauna, overlying a phosphate epigenised foraminiferal chalk of unknown age. This suggests that the last eruptive phase recorded in the sampling area occurred 45 Myr BP, and gave rise to fractionated volcanics of an alkaline nature.

Fig. 2 A profile drawn from a multichannel narrow beam survey chart made in the sampled area located near station CH 77-07 on the Sierra Leone Rise.



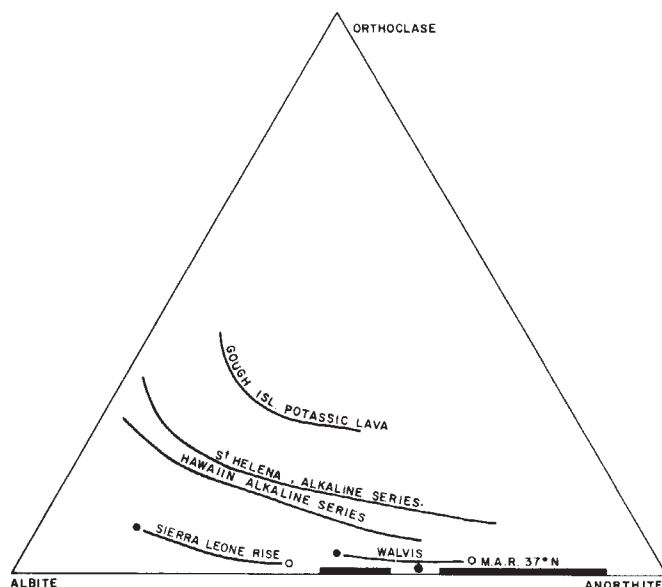


Fig. 3 An-or-ab ternary diagram of feldspar composition from various localities. The data from Gough island¹⁵, from Hawaii¹⁶ and from St Helena¹⁷ we compared with that from the Walvis Ridge and the Sierra Leone Rise. An open circle indicates phenocrysts of analysed feldspar and the filled circles are matrix feldspar found in the corresponding rock samples. The data on the plagioclase from near 37° N in the Atlantic ocean were taken from basalts of the inner floor¹¹ and those from the Rift Mountain Region¹² of the Rift Valley.

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An ophiolite complex of probable early Caledonian age discovered on Karmøy

THE existence, in the Lower Palaeozoic sequence of western Norway, of a major ophiolite complex which is outlined here is shown best on the island of Karmøy, western Norway. Our preliminary interpretation is that the ophiolite complex was thrust into position during the Grampian phase of the Caledonian orogeny, although it has probably been subsequently displaced during the end-Silurian phase of that orogeny.

It is clearly established that the allochthonous Caledonian sequence of western Norway consists of two major complexes, with different tectono-metamorphic evolution, separated by a major stratigraphic unconformity of middle/upper Ordovician age¹⁻³. The older complex shows the more involved tectono-metamorphic history and in many areas was subjected to strong retrogression during the end-Silurian phase of the Caledonian orogeny. The main metamorphism and deformation of this older complex has been correlated with the Grampian deformation in the Scottish and Irish Dalradian^{3,9}. Within the older sequence, thick piles of submarine metabasalts are widely developed. They show pronounced geochemical similarities to ocean-floor basalts, and have been presumed to relate to ridge spreading in a proto-Atlantic ocean⁴⁻⁷. The metabasaltic sequences may be of considerable thickness and in many areas are associated with intrusive cumulate gabbros, trondhjemitic and minor developments of serpentinised peridotites. This has given rise to speculations on the possible ophiolitic affinities of the assemblage⁸. However, none of the previously described sequences demonstrate a fully developed ophiolite stratigraphy. Indeed, in many areas, such as the Bergen Arcs^{7,9}, the thick submarine metabasalts and their associated intrusive rocks lie stratigraphically above a considerable thickness of metasediments.

Our recent investigations in the Karmøy region have revealed the presence of a cross section through a major early Caledonian ophiolite complex, which we call the Karmøy Ophiolite. The basal contact of the ophiolite is exposed on the Haugesund peninsula and represents a low angle thrust. On Karmøy the ophiolite sequence is truncated by a major stratigraphic unconformity (Figs 1 and 2) beneath the Skudeneshet Group of middle/upper Ordovician age. The ophiolite contains a tripartite stratigraphy in ascending order as follows.

Zone III: a lowermost plutonic zone comprising layered gabbroic and ultrabasic intrusions together with minor developments of trondhjemitic, and cut by later basic dykes. The gabbroic and ultrabasic rocks represent a polyphasic series of intrusions and are in many cases well banded cumulates. Observations of structures in the cumulate sequence show consistent upward younging. These rocks and the associated trondhjemitic intrusions contain evidence of successive phases of high temperature deformation before the emplacement of a major linear steeply inclined dyke swarm of metabasalts and diabases which extends into the overlying submarine metabasaltic pile. This pattern, of pre-dyke deformation is analogous with described occurrences from the Oman ophiolite¹⁰, and the Bay of Islands ophiolite¹¹⁻¹³. At the top of this plutonic zone the dyke swarm becomes a sheeted dyke complex, well exposed at the Airport, and we refer to this as the Lufthavnen sheeted dyke complex. This attains in places 100% dyke rock with only minor disconnected screens of wall rock. These screens are of gabbro in the lower part of the sheeted dyke complex. Screens of gabbro are not found in the upper parts, but gradually it becomes possible, as the sheeted dykes become less dense, to distinguish screens of pillowed metabasalt. In places, however, the contact with the overlying pillow sequence is complicated by later faulting. The dyke swarm observed in the plutonic zone increases in density up towards the true sheeted dyke complex.

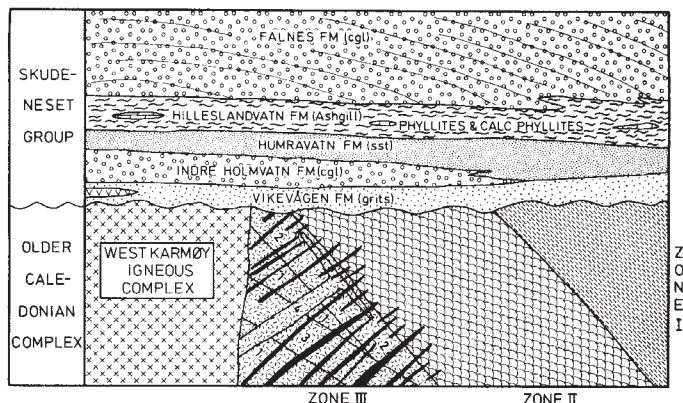
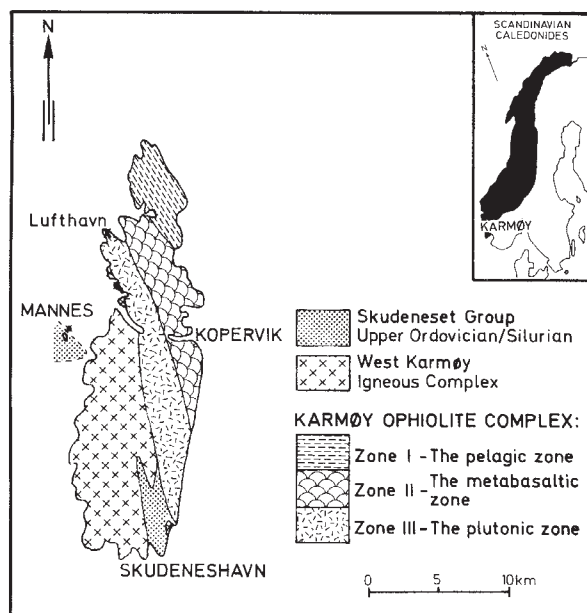


Fig. 1 Simplified geological map of Karmøy showing distribution of major units.

Zone II: above this a >2 km thick sequence of greenstones occurs which contains both pillowed and massive flow units of metabasalt, with irregularly developed hyaloclastites. The rocks within this zone show varying intensities of deformation, ultimately producing greenschists from the basalts, although where way-up structures have been observed they show consistent younging away from the lower plutonic zone III. In the upper part of the metabasalt column, horizons of greenschists, which may represent volcanoclastic materials, and irregular jasper layers and bedded cherts are intercalated with metabasalts. Dyke rocks similar to those observed in zone III also cut the metabasalt sequence, but diminish in intensity upwards. Several examples have been observed where such dykes feed individual flow units, and we conclude that the dyke swarm represents the feeders to the lava pile.

Zone I: overlying the metabasalts of zone II, and preserved in a large secondary synform, there occurs a sequence, several hundred metres thick, consisting of ribbon cherts, bedded jasperites, black schists, greenschists which may be of volcanoclastic origin, minor calcareous horizons and in the lower part, thin flow units of pillowed basalts. The main schistosity is related to upright F_1 folds with subhorizontal northwesterly trending axis and an associated steeply dipping northwesterly

Fig. 2 Idealised profile illustrating the relationships of the Karmøy Ophiolite; the rocks of the Skudenese Group, though folded, are drawn horizontal. In zone III, nos 1–4 indicate the polyphasic nature of the plutonic assemblage.



trending axial planar schistosity. The rocks of zone I are considered to represent a series of pelagic sediments.

We consider that the zonal sequence I–III described above represents, respectively, layers 1, 2 and 3 of a typical cross-section through oceanic crust. Massive sulphide deposits (Cu, Ni) occur on Karmøy and the neighbouring small islands, though as yet no podiform chromites have been observed in the serpentinised peridotites.

It is not possible, at this stage to provide a precise age for the thrusting (obduction) of the Karmøy Ophiolite on to the Baltic Shield, although by analogy with other Caledonian ophiolite complexes in the North Atlantic region, that is, the Girvan¹⁴ and Newfoundland complexes¹⁴, it may be of lower Ordovician age. The Karmøy Ophiolite, having been both deformed and metamorphosed in greenschist facies, was then intruded by the rocks of the West Karmøy Igneous Complex¹⁵. This latter is a late syntectonic complex of granitic and dioritic rocks with at least five major phases of intrusion. Preliminary Rb/Sr whole rock isochron studies indicate that late granodioritic dykes have an age of ~450 Myr ($\lambda^{87}\text{Rb} = 1.39 \cdot 10^{-11} \text{ yr}^{-1}$) with an initial $^{87}\text{Sr}/^{86}\text{Sr}$ of 0.7210. The obduction of the Karmøy Ophiolite, on to the Baltic Shield, is considered to have occurred before the emplacement of the West Karmøy Igneous Complex, and thus, the date obtained for the granodioritic dykes provides a minimum age for ophiolite obduction. The presence of highly assimilated gneiss xenoliths in some of the granitic rocks, and the high initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of the granodioritic dykes, implies a continental crustal source for the West Karmøy Igneous Complex, beneath the ophiolite. It is thus speculated that the granitic and dioritic magmas originated through partial melting in the downgoing continental plate of Baltic Shield crystallines, perhaps in relation to the active translation of the ophiolite slab.

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The source of chert for aboriginal artefacts in southwestern Australia

ABORIGINAL chert artefacts abound in surface assemblages along the western margin of southwestern Australia north of Perth, but no local source of chert is known. The decrease in proportion of chert in assemblages further from the coast has been used as evidence of a westerly source on the present continental shelf but no data have been provided to show that this source is practical. Recent drilling by oil companies offshore in the northern Perth Basin has now shown that chert occurs at depths within reach of the last glacial sea level low and that the postulated source is not only reasonable but highly probable.

Chert is favoured over other materials because of its hardness, and conchoidal fracture which make working of edges and

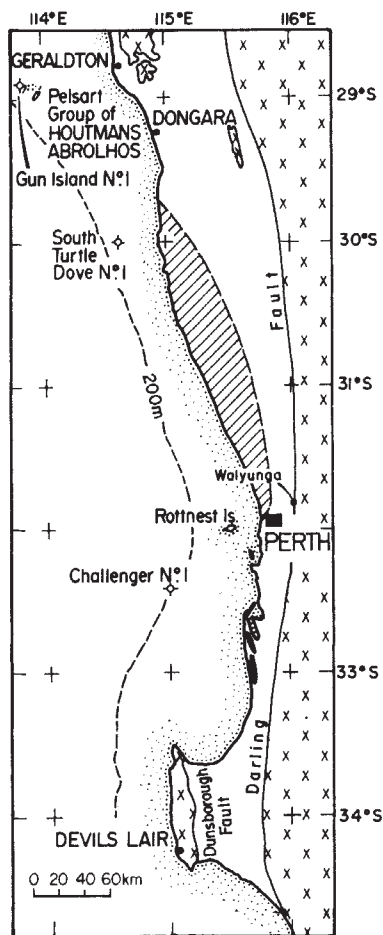


Fig. 1 Occurrence of Eocene chert artefacts (cross hatched area) in relation to the Precambrian Shield area (crosses) in southwestern Australia.

points simple, and because the products are durable. Several recent papers¹⁻⁶ have discussed the distribution of the artefacts including the aspect of source because no similar chert is known in outcrop in the Perth Basin. Glover and Cockbain¹ showed that the chert is of Middle and/or Late Eocene age on the basis of the characteristic benthic foraminiferid *Maslinella chapmani* Glaessner and Wade.

Chert, identical to that found north of Perth, occurs as artefacts in sediment dated at 12,000–17,000 yr BP from the excavations at Devils Lair on the southwestern tip of Western Australia² and more recently⁵, chert as recent as perhaps 4,500 yr BP has been discovered at Walyunga, close to Perth. The former age in particular corresponds closely with the last glacially induced sea level low estimated at about 105 m below modern sea level⁷, after allowing for hydroisostatic depression of the continental margin. New data have become available from examination of sediments from four wells offshore in the Perth Basin (Fig. 1)—Challenger No. 1 (WAPET, 1975)⁸, Rottne Island Bore (W.A. Govt. 1913)⁸, South Turtle Dove No. 1 (WAPET, 1975) and particularly Gun Island No. 1 (BP-1968)⁹—whose Cainozoic stratigraphic sections are shown on Fig. 2. Challenger No. 1 and Gun Island No. 1 both contain Late Eocene sections of cherty limestone of P15/P16^{10,11} age sediments, that from Gun Island also containing *Maslinella chapmani*. Rottne Island Bore contains a Middle Eocene section^{8,12,13} without chert but South Turtle Dove has Middle Eocene limestone with chert equivalent in age to sediments in the Rottne Island Bore. The Middle Eocene sediments in the Rottne Island Bore and South Turtle Dove No. 1 represent deposition during a Middle Eocene interval of high sea level, a

different transgression from the Late Eocene¹⁰ sea level high indicated by the sediments in the other two wells and elsewhere⁸. Thus the most likely source of artefacts, if outcrop occurred, would be the Late Eocene unit, although Middle Eocene cherty limestone cannot be ruled out as an additional source.

The most significant section is that encountered in Gun Island No. 1. The uppermost sample⁹ recovered from this well was a core (Core No. 1) which contains an excellently preserved fauna of Late Eocene planktonic foraminiferids, including *Globigerinatheka index index* (Finlay), *Globorotalia cerroazulensis* (Cole), *Globigerina tapuriensis* Blow and Banner, *G. gortanii praeturritilina* Blow and Banner, *G. utilisindex* Jenkins and Orr, and *Pseudogloboquadrina primitiva* (Finlay) which indicate a Late Eocene age (P15/P16)¹¹. This material was originally identified as Late Miocene in age⁹. It occurs at 131 m below sea level and probably extends higher. The base of the Late Eocene section is at 228 or 252 m below sea level, the doubt hinging on the age of two undated samples at 246 and 252 m below sea level. Somewhere above the Eocene section is some Miocene,

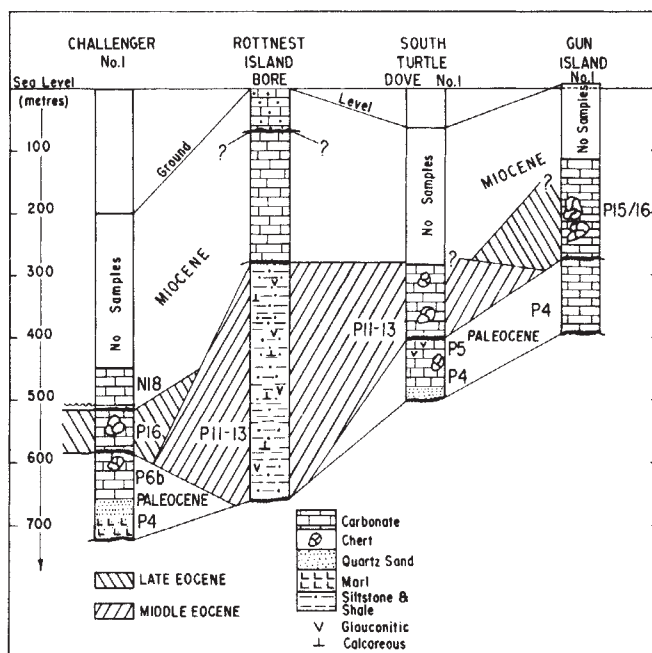


Fig. 2 Perth Basin sections with Middle–Late Eocene rocks.

required to explain the presence of *Lepidocyclus* in cutting samples from deeper in the well.

In consequence of the discovery of Middle–Late Eocene cherty sediments in several localities west of the present coastline, and at depths within reach of known Quaternary sea level depressions, it is clear that outcrops of these sediments occurred on the present inner continental shelf during the last glacial sea level low and that these provided the source of chert for aboriginal man's artefacts. A westerly source is also indicated by the higher proportion of chert in artefact collections taken from near the present coastline, the proportion decreasing eastward⁴. A westerly source has been suggested^{4,8} and the results of drilling Challenger No. 1 reported⁸, but the results from Gun Island No. 1 are much more relevant to the problem and convincing in proving the source. Searching the continental shelf of Australia and elsewhere will reveal other evidence of pre-historic man's presence. As the Eocene is recognised as an epoch whose sediments commonly contain chert¹⁴, it behoves scientists working on passive continental margins, particularly adjacent to areas of low rainfall and runoff, such as southwest Africa, to be aware of the possibility of the outcrop of Eocene chert during

intervals of low sea level, and of finding evidence of prehistoric human habitation on the present shelf during times of low sea level.

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Why do we not perceive photons?

VERY few photons need to be captured by the receptors of the human eye to excite a sensation of light. The number required is certainly no greater than 100 and may be much less^{1,2}. A single rod requires only one photon to signal light³. A question which therefore arises is why do we not see punctate photon absorptions when we look at a very dim patch of light? The retina catches incident photons only sporadically in time, and therefore in space, when a source is very dim. If very few photons are sufficient to detect light, it might be expected that we should see isolated flashes of light, so that a patch of light appears mottled or speckled. Indeed, if a dim object is viewed on the screen of a cascaded image intensifier, so that each quantum caught by the cathode is amplified, we do see quanta as individual flashes or specks on the display phosphor². This apparent contradiction creates a physiological problem ignored in most standard textbooks on vision although the issue has been raised^{4,5}. We report here a possible solution; we have observed point sources of light set up one at a time very rapidly and at random by computer within a square patch on the face of an oscilloscope and find that as the luminance of the point sources is decreased the number of specks seen increases markedly, and that the appearance of motion, due presumably in the first place to the effect of the chance sequences of specks on motion-sensitive neurones, becomes weaker. We propose that at or near the threshold, the number of specks seen increases to infinity so that the specks crowd together to appear uniform and so the motion ceases.

To test this, after becoming adapted to the dark, we viewed the oscilloscope through a neutral density wedge. Although persistence on the oscilloscope phosphor is brief, a collection of points of changing distribution but constant mean number appears continuously in view. This reflects the operation of storage mechanisms in vision which prolong the visibility of each speck. The number of specks visible to the observer provides a measure of the time constants involved. In a dark room the wedge filter was first set so that nothing was visible. Then it was adjusted until the shape of the square patch on the screen was just continuously discernible. At this level of luminance it was also possible to identify correctly dark shapes (a circle, a square or a triangle, each of which was one-quarter the area of the basic

square patch) in front of the screen. The display looked uniform and devoid of motion, although no more than one light point was being displayed on the screen at any instant.

Now a uniform patch was generated filling the area where the point display had been. A switch enabled us to interchange the point and uniform display. At the threshold for shape identification, the uniform patch could not be distinguished from the random point display.

If the luminance of the point display was again increased, mottled blobs were seen moving around sluggishly; no individual points could be resolved. The display then looked quite different from the uniform display at any luminance. A further small increase in luminance changed the appearance of the patch to a very large number of small discrete points each agitatedly jumping about. The transition from mottled blobs to sharp discrete points occurred over a small range of luminance. As the luminance is raised further to very high levels, the number of specks perceived decreases, each speck appears brighter, and movement becomes less agitated.

These changes in number, motion and texture are so dramatic that a naive observer is astonished that nothing about the display has changed except the luminance, over which he has personal control.

In further experiment, points were plotted at random in a rectangular patch, but every few seconds, four points, arranged to form a square, were displayed, each once only, alongside the rectangular patch. The four points were each of the same luminance as those plotted at random within the rectangular patch. As the luminance decreased the isolated four points were visible only sporadically. Sometimes all four might be seen, sometimes none were seen, and sometimes a random subset was seen. The rectangular patch was seen as uniform only if we could never see any one of the four isolated points.

This finding implies that points are additive in their effects at the luminance levels where the patch presents a uniform appearance. Individual points on their own emit too few photons ever to be visible as specks. Persistence of individual points, perceived as specks, therefore does not explain the impression of uniformity: another effect is involved. A clue to this additional effect is provided by close observation of the four isolated points near threshold. Each point, if visible at all, varies on each appearance, in size, from a small spot to a large globule, and in orientation, sometimes being elongated along one axis, sometimes along another.

Pirenne and Denton³ suggested that at the threshold for vision, the number of quanta required for a sensation of light may be the same for detectors which respond to the activity of different groups of receptors. If so, it will be those detectors with a greater catchment network of receptors which will be most likely to respond when the light is least. It is also these same detectors which are most likely to respond to quanta caught from two or more point sources in different places, as they encompass a larger number of receptors. The functional organisation of the retina will seem to change⁶, when, in reality, all that has happened is that certain detectors are now favoured by the circumstance of occasional quantum emission.

Considerable evidence, both psychophysical and neurophysiological, suggests that vision is organised into channels⁷ specialised for size, and, independently, orientation⁸. Our observation of variation in appearance of single points of light near threshold is consistent with the assumption that sometimes one, and sometimes another, of such channels are by chance obtaining a sufficient quantum catch to report on the appearance of a single spot of light. An analogous effect of variable appearance in the orientation of short lines was reported by Andrews⁹. Those channels which report a larger size are, of course, favoured.

The appearance of uniformity both of a uniform surface in clear light and of a surface made of kinetic points appearing at random, seems to be the combined result of two effects. One is a prolongation of the impression made by quanta caught in either case. The other is a consequence of the organisation of the visual

system into detectors arranged to catch larger patterns of light as the number of quanta caught becomes more sparse. It is perhaps fortunate that our visual system has been so designed as to present uniform surfaces to us as uniform at these low photon levels. If this were not so, surfaces would dissolve in the night into collections of scintillating spots, and the shower of photons from the dim night-sky would obscure the perception of the star formations.

An incidental observation was that any of the four individual points in the last experiment could seem to vary in depth, as well as in size and orientation, when observed with both eyes. This effect is presumably due to random stimulation of different disparity detectors at these low light levels.

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On feeding on more than one trophic level

IN trying to understand the structure of ecological communities, ecologists usually pay particular attention to the interactions between pairs, or small groups of species¹. Questions about the 'shape' of the food webs within which these species are embedded are much more rarely asked^{2–4}. For example, what happens when a population feeds at more than one trophic level (omnivory)? In some real food webs there seem to be no omnivores (Fig. 1a)⁵; in others omnivores are common^{6,7} (Fig. 1c)⁸. In this note we attack the problem of omnivory using simple, linear Lotka–Volterra models of food webs⁹, and show that certain patterns are much more likely to persist on an evolutionary time scale than others. We then compare the model predictions with real food webs.

Consider a system of n interacting species described by equations of the form

$$\frac{dX_i}{dt} = X_i \left(b_i + \sum_{j=1}^n a_{ij} X_j \right) \quad (1)$$

where X_i is the population size and b_i the instantaneous rate of growth of the i th species, and a_{ij} is the *per capita* effect of the j th species on the i th species. We used equation (1) to construct model food webs, using as our criterion for realism the notion that ecologically feasible models must be locally stable^{1,3,9}, and that a rapid return to equilibrium enhances stability^{9,10}.

For models with four species and four trophic levels there are eight possible configurations (Fig. 2). These are distinguished by the 'rank of omnivory', which is the number of predator–prey interactions in excess of those in the model with no omnivory, and by the position of these interactions. Matrices, with sign configurations corresponding to these models were analysed

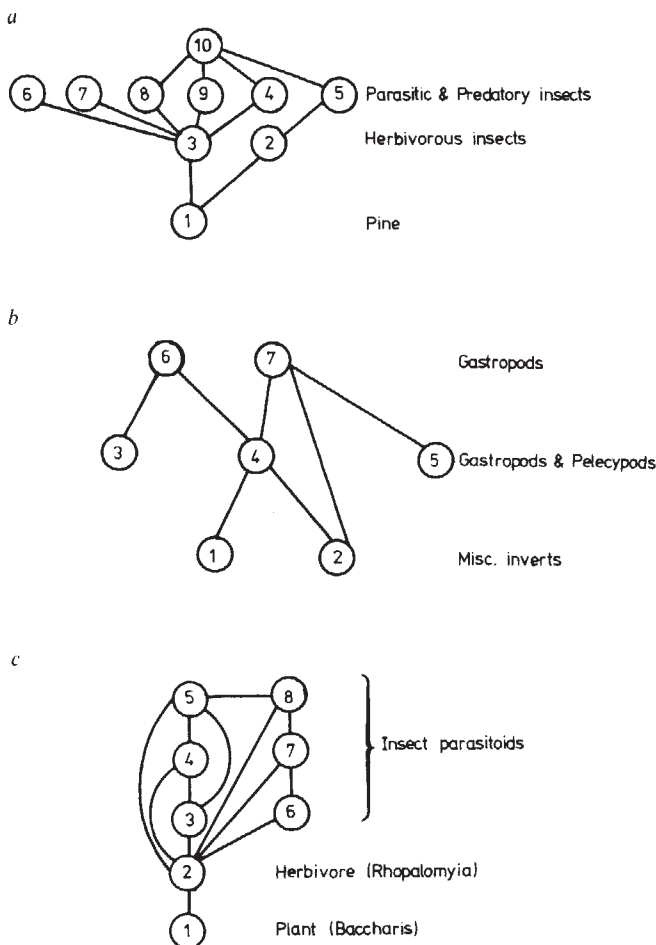
numerically to determine their maximum eigenvalues ($\lambda_{\max}$), their stability [$\text{Real}(\lambda_{\max}) < 0$] and their relative return times to equilibrium, RT , defined by

$$RT \equiv -\frac{1}{\text{Real}(\lambda_{\max})} \quad \lambda_{\max} < 0$$

The elements of the matrices were chosen uniformly at random with 1,000 replications over three sets of parameter limits, labelled 'vertebrate', 'vertebrate/insect', and 'parasitoid', respectively.

(1) Vertebrate: The off-diagonal elements of the matrices are the products of the *per capita* effect of the prey on the predator multiplied by the predator's equilibrium density or the *per capita* effect of the predator on the prey multiplied by the prey's equilibrium density ($a_{ij}X_i^*$). When predators are larger than their prey the effect of the predator on the prey will be much larger than the converse and the predator's density will be smaller than that of its prey. The elements corresponding to the predator's effect on the prey will therefore be of much greater absolute value than the converse; we chose values on the interval of $(-10, 0)$ for the former and $(0, +0.1)$ for the latter for this set of models. (Note that very large, invertebrate predators (such as starfish) and their prey will also fall into this category.)

Fig. 1 Diagrammatic representation of three natural food webs. *a*, Relationships between insects feeding on Pine⁵. *b*, A simplified representation of the interactions between intertidal gastropods, pelecypods and their prey⁶. Interactions accounting for less than 20% of the predator's diet have been omitted; without this simplification omnivory is common in this web. *c*, Part of a simplified insect host–parasitoid web on a single food plant⁸. Omnivory is common in such webs.



(2) Vertebrate/insect: The *per capita* effect of an insect feeding on a tree is very small, the converse very large. For this set of simulations we chose the interval of the effect of trophic level one on two to be (0, +10) and the converse to be (-0.1, 0). The remainder of the interactions were as (1).

(3) Parasitoid: Here, interactions between trophic levels one and two were as in (2). The remainder were chosen to mimic insect host-parasitoid interactions¹¹, in which the equilibrium population sizes of host and parasitoid may be more similar than in (1), and the *per capita* effect of the host on the parasitoid is large (one host frequently gives rise to one parasitoid). The reciprocal interactions were therefore assigned similar magnitudes, on the intervals: parasitoid on host (-1, 0), host on parasitoid (0, +1).

In all the models, the diagonal elements for the heterotrophic species were zero, implying that each species in the chain (except the first) was ultimately limited only by the abundance of food in the preceding trophic level and not, for example, by behavioral conventions^{12,13}. The species at the base of each food chain (plants) were self limited, with coefficients distributed on the interval (-1, 0).

The option of choosing Jacobian entries directly over the more frequent choice of the b_i and the a_{ij} has several advantages. Rates of increase and decrease in the absence of all other species (b_i) are very difficult for us to visualise; the equilibrium populations (X_i^*) and the effects of predators on prey and vice versa (a_{ij}) are much more obvious. Our models necessarily have positive X_i^* , as the Jacobian entries and the a_{ij} have exactly the same signs. Thus we do not need to calculate the X_i^* from the b_i and the a_{ij} , check their positivity and then calculate the Jacobian; the savings in computation are quite considerable.

For the three sets of eight models, the percentage of unstable models and those stable models with return times in excess of 100 are shown in Table 1. Note first that as the rank of omnivory increases, the percentage of unstable models increases, but for the subsets that are stable, the percentage with long return times generally decreases. These effects work in opposition to one another, so that in the real world, feasibility could either increase or decrease with rank. However, for the vertebrate and vertebrate/insect models, very few matrices were stable for rank greater than one. Second, models of rank one differ in their feasibility, depending on how many levels are spanned by the predator. While over 9% of the vertebrate and vertebrate/insect models are stable when the predator feeds on levels one and two, or two and three, less than 4% of the models are

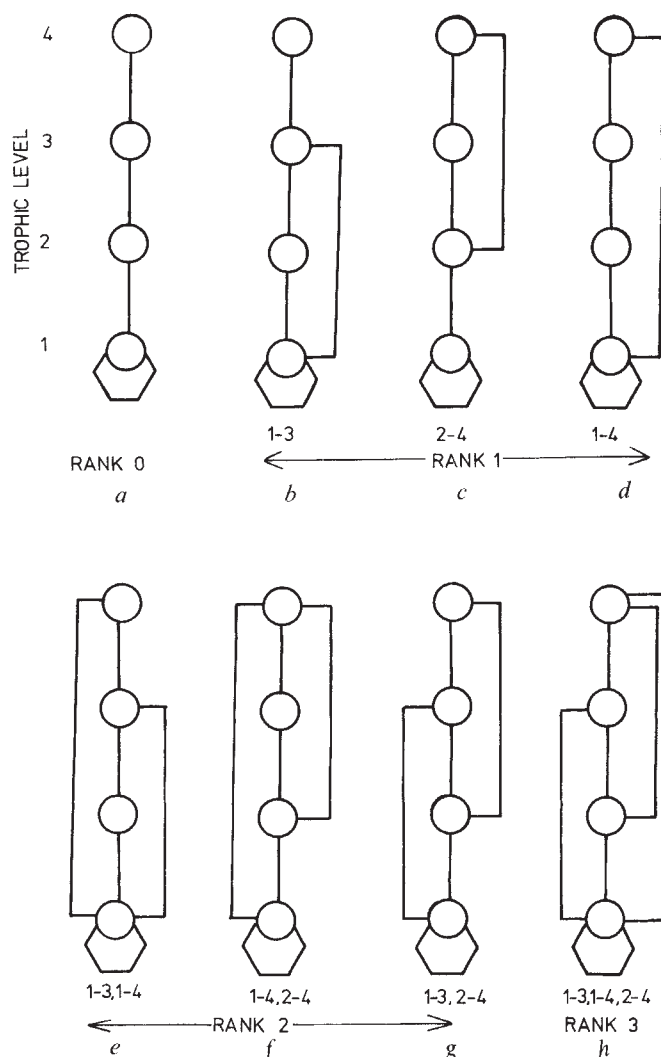


Fig. 2 Theoretical food-chains. *a*, Without omnivores; *b-d*, with one omnivore-link; *e-g*, with two and *h*, with three omnivore links. The species at the base of each food chain (plants, level 1) are self-limiting. These food chains can be modelled using equations of the form specified by equation (1).

Table 1 Stability of 1,000 random food webs of each type, constructed according to the range of parameter values specified in the text

Percentages of unstable models		Models		
Rank of omnivory	Position (species linked)	Vertebrate	Vertebrate/insect	Parasitoid
0	—	0	0	0
1	1-3	81	78	40
	2-4	91	91	68
	1-4	96	96	51
2	1-4 & 2-4	98	98	59
	1-3 & 2-4	97	97	74
	1-4 & 1-3	99	99	50
3	1-3, 1-4, & 2-4	100	100	72
Percentages of stable models with return times > 100				
0	—	44	41	42
1	1-3	21	22	19
	2-4	11	13	19
	1-4	47	46	38
2	1-4 & 2-4	—	—	11
	1-3 & 2-4	—	—	13
	1-4 & 1-3	—	—	13
3	1-3, 2-4, 1-4	—	—	13

Percentages are given to the nearest integer.

stable where a predator feeds on levels one and three. In these latter models a high percentage also have long return times. Third and last, although vertebrate and vertebrate/insect webs show almost identical patterns, the parasitoid webs are generally much more stable though their return times are comparable. High ranking omnivory is relatively easily attained in these webs.

Increasing the rank of omnivory in these models also increases connectance with its well known effect on decreasing stability. However, it is important to investigate the precise restrictions on web shape that low connectance implies. We show elsewhere¹⁴ that our results still hold if the effects of increasing omnivory and connectance are changed independently.

Bringing these results together, we predict that webs with large numbers of omnivores (high ranking omnivory) will be rare in the real world, except in insect host-parasitoid systems (or other groups of species with comparable interaction coefficients and equilibrium population sizes). We also predict that it will be extremely rare to find species that feed simultaneously both high and low in a food web.

Using data on 19 food webs from tropical, temperate, aquatic and terrestrial systems accumulated by Cohen¹⁵ we examined 58 food chains with three or more trophic levels. Of these, 24 had no omnivores, 23 omnivory of rank one, and 11 of rank two or

more. At first sight, 11 webs with high-ranking omnivory is more than might be expected on the basis of our models. However, an examination of the 11 webs showed that in three cases the omnivory was associated with gross lumping of categories (invertebrates, insects and zooplankton), and hence is probably an artefact of the data (because species that are otherwise similar but which feed at different trophic levels are grouped together). Consideration of the remaining webs also highlights a more fundamental problem—the difficulty of distinguishing between links in real food webs that significantly depress the populations being fed upon (controlling links, as in the models) from those that do not. Inclusion of the latter inevitably leads to omnivory (and connectance in general³) being overestimated. This may be compensated for in part by discounting links that provide only a small percentage of an omnivore's diet. Thus, omitting links that make up less than 20% of the prey items taken by omnivores in Paine's⁶ study of a very complex intertidal community, yields a simplified web with only one omnivore (Fig. 1b). Similar results are obtained for the other communities studied by Paine¹⁶. Unfortunately, there are no data available which permit us to analyse the remaining food webs with high ranking omnivory in the same way, and we cannot tell what proportion of the omnivorous links in these webs is also important.

These difficulties aside, our other predictions seem to be supported by the data. Thus, of the 23 chains with omnivory of rank one, only one involves a species which feeds on trophic-levels one (plant) and three (animal) (as in Fig. 2d). The remainder feed on consecutive trophic levels, either one and two (Fig. 2b) or two and three (Fig. 2c). This distribution is significantly different from random ($P < 0.01$), and supports our second prediction. [Of the 65 omnivores examined, 30 fed on levels two and three (both animal) and 35 fed on levels one and two, suggesting that dissimilarity in diet alone is not sufficient to explain the rarity of 1–3 links.]

Only one of Cohen's webs involves insects⁵, and it was one of the simplest analysed (Fig. 1a). However, several highly complex insect-parasitoid webs have also been reported^{7,8}, and it is at least encouraging to see very complex webs developing in the real world in exactly those situations which our models predict are feasible. Figure 1c shows an example⁸. Even after making allowance for the fact that some of the links are minor, such webs tend to be more complex than nonparasitoid webs. Facultative hyperparasitism (omnivory) is particularly common in this group of animals^{7,17,18}.

Obviously there are weaknesses and omissions in most of the published data on food webs, and there are reasons other than population dynamics making omnivory difficult. For example, some species must be prevented from feeding at more than one trophic level by the markedly dissimilar types of food found in each, and some of the differences that we have observed between vertebrate and parasitoid webs may arise because of the peculiar mode of nutrition adopted by insect parasitoids. Comparisons between vertebrate and insect webs are also biased by the tendency for vertebrate ecologists to exclude true parasites from consideration. (When a fox eats a rabbit, it also eats the rabbit's parasites.) This is an unfortunate omission, because internal parasites influence the dynamics of their hosts far more than has generally been realised^{19,20}.

Omnivory in the way that we have modelled it can be generated by several different biological processes: by individuals which seek out prey from different trophic levels at one stage in their life history (conventional omnivory); by different age-classes of one species feeding on different foods (or hosts in the case of true parasites (R. M. Anderson, personal communication)) and by individuals consuming prey and its internal parasites 'in the same mouthful'. But irrespective of the mechanism by which they are generated, trophic links must satisfy certain dynamical constraints if the resulting food webs are to persist. Our models suggest that some trophic patterns and combinations of parameter values are much more stable than others. We believe that considerations of this kind may have an important, but neglected role in determining whether

particular species or whole sets of species can coexist in ecological systems, and points the way to a more general understanding of the design of natural food webs.

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Competition among desert perennials

THE accurate description of horizontal patterns of distribution and the determination of the mechanisms resulting in these patterns^{1,2} are primary objectives of plant ecology. Although there have been many studies of horizontal patterns, most investigations have not proceeded beyond the initial stage of detection and analysis of pattern, leaving suggestions of causation untested. Controlled field experiments that test mechanisms which may regulate distributions are rare³. We have designed a controlled field experiment to evaluate the effects of intra- and interspecific interactions affecting two distinct horizontal patterns, thereby providing a test of current plant distribution theory.

We selected a 2.5-hectare area in the Mojave Desert near Lucerne Valley, San Bernardino County, California (34°33' N, 116°53' W). The terrain is level, ensuring even deposition of rainfall, and consists of deep granitic alluvium. The vegetation is co-dominated by *Larrea tridentata* Cav. (Zygophyllaceae) and *Ambrosia dumosa* (Gray) Payne (Asteraceae) with derived species' importance values (based on their relative dominance, relative frequency and relative density in 104 5 m × 5 m random quadrats) of 39.7% and 49.2%, respectively.

Both these species are important components of many desert communities—co-dominating 70 per cent of the Mojave Desert⁴. *Ambrosia* is short, hemispherically shaped and drought-deciduous, whereas *Larrea* is tall, cone-shaped and evergreen. Also, *Larrea* has been shown to be regularly distributed in certain areas of both the Mojave and Sonoran Deserts^{5,6}, where competition for moisture is hypothetically very keen.

To determine the horizontal distribution of these species, we sampled the site and analysed our data in a manner similar to that of Woodell *et al.*⁵. The density of *Larrea* and *Ambrosia* were measured in 250 10 m × 10 m quadrats from a random,

Table 1 Horizontal distribution of shrubs

Species	Mean ($\bar{x}$) (shrubs per 100 m ²)	Variance (σ^2)	$\frac{\sigma^2}{\bar{x}}$	Distribution
<i>Larrea tridentata</i>	3.62	2.06	0.57	Regular
<i>Ambrosia dumosa</i>	19.52	96.56	4.95	Contagious

Both species' distributions are significantly nonrandom at the 0.1% level using Student's *t* test.

stratified sample. Utilising the variance/mean ratio method described by Greig-Smith¹ (significantly >1 = clumped, significantly <1 = regular), we determined the direction and degree of non randomness. The results in Table 1 clearly indicate that the horizontal distribution of *Ambrosia* is clumped and that of *Larrea* is regular. Using Pearson product-moment linear correlations of the density and the total biomass per 5 m $\times$ 5 m quadrat of *Ambrosia* on *Larrea* (which proved insignificant), we concluded that *Larrea* and *Ambrosia* were independently distributed relative to each other⁷.

As all regular and some clumped distributions have been attributed to plant-plant interactions and because the literature suggests the occurrence of competition for moisture in the desert^{2,5,8,9,10}, we decided to investigate these possible distributional control mechanisms using a differential removal experiment in the field. Forty 100²-m circular plots (5.64 m radius) within the 2.5-hectare area were circumscribed using average-sized *Larrea* bushes for centre points and as monitored plants. From these plots four categories (10 replicates each) of control and test plots were randomly chosen. These categories were: (1) control—no plants removed; (2) total removal—all *Larrea* and *Ambrosia* except centre *Larrea* removed; (3) *Larrea* removal—all *Larrea* except centre *Larrea* removed; (4) *Ambrosia* removal—all *Ambrosia* removed. Plants were removed by cutting at ground level. We also set out four equivalent categories of plots in which individuals of *Ambrosia* were similarly utilised as both centre points for circumscribing and for monitoring. On completion of the various treatments, predawn measurements of plant water potential were taken to evaluate the water status of the monitored plants using the Scholander pressure bomb method^{11,12}.

Immediately after a 60-mm rainfall in August, we began to measure water potentials at semi-monthly intervals. Initially the water potentials of *Larrea* were ~ -20 bar and those of *Ambrosia* ~ -2 bar with no consistent differences among treatments. (These are high potentials for both species.) As drying of the soil continued and plant water potentials decreased through time, consistent differences among treatments developed (Table 2). In the *Larrea*-monitored plots the mean water potential of the monitored *Larrea* plants in the control

plots (-39.0 bar) was significantly lower than that of the *Larrea* plants in the total removal (-33.7 bar) and in the *Ambrosia* removal plots (-34.6 bar), but not significantly different from the mean water potential of the monitored plants in the *Larrea* removal plots (-39.3 bar). Similar results were obtained in the *Ambrosia*-monitored plots. The mean water potential of the monitored *Ambrosia* plants in the control plots (-44.2 bar) was significantly lower than that in the total removal plots (-30.5 bar) and in the *Larrea* removal plots (-36.5 bar), but not significantly different from the mean water potential of the monitored plants in the *Ambrosia* removal plots (-44.6 bar).

To our knowledge this is the first experimental evaluation of plant competition in deserts. The results of this study suggest that competition for soil moisture does occur at this site when water availability is low and that, with the present horizontal arrangement of individuals, interspecific competition is more intense than intraspecific competition. The actual mechanism(s) responsible for the interspecific effects on plant water potentials is undetermined. While we suspect the mechanism may be a simple depletion of a limiting resource (water) by interacting species, the possibility of allelopathy, especially by the roots, cannot be ruled out.

Furthermore, it must be emphasised that, because of the feedback type of relationship between competition and distribution, the present status of competition at this desert site may not be indicative of the historical interactions among the plants which have resulted in the present distributions. It is possible that at our site *Larrea* plants were once clumped and competed intensely with each other. Through time, such competition may have resulted in the present regular distribution in which few plants are close to each other, and competition is thereby minimised. Alternatively, *Ambrosia* plants may have remained clumped because most of their growth occurs at relatively high water potentials (>-30 bar) when water may not be a limiting resource.

The negative interactions between *Larrea* and *Ambrosia*, indicated by the plant water potentials in this study, are surprising in view of the independent distributions of these species relative to each other. These distributions may be the result of other, overriding, physiological and environmental factors, or the interspecific interactions have not been of sufficient intensity or duration to affect the distributions. The significance of the negative interactions between *Larrea* and *Ambrosia* is yet to be determined in terms of production.

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Table 2 Differential effects of removal treatments on plant water potentials

<i>Larrea</i>		<i>Ambrosia</i>	
Treatment	Water potential (bar)	Treatment	Water potential (bar)
a Control	$-39.0 \pm 1.3^{b,d}$	a Control	$-44.2 \pm 3.2^{b,d}$
b Total removal	$-33.7 \pm 1.6^{a,c}$	b Total removal	$-30.5 \pm 3.4^{a,c,d}$
c <i>Larrea</i> removal	$-39.3 \pm 2.3^{b,d}$	c <i>Ambrosia</i> removal	$-44.6 \pm 3.9^{b,d}$
d <i>Ambrosia</i> removal	$-34.6 \pm 2.7^{a,c}$	d <i>Larrea</i> removal	$-36.5 \pm 2.8^{a,b,c}$

Small letters in data column denote statistical differences from the treatments (a = control; b = total removal; c = intraspecific removal; d = interspecific removal) at $P < 0.05$ using Student's *t* test. The water potential data (14 November 1977) are based on five randomly chosen plants (three twigs per plant) per treatment.

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Diverse modes of action of biotic and abiotic phytoalexin elicitors

PHYTOALEXINS are inducibly formed higher plant metabolites that are antibiotic to certain potential plant pathogens¹. At least 75 plant species representing 20 families have been shown to accumulate phytoalexins in response to infection¹⁻³. Phytoalexins also accumulate in plants in response to various agents termed elicitors¹, including substances of pathogen origin (biotic elicitors) and abiotic elicitors such as heavy metal salts and detergents¹⁻³. Elicitors may be useful for investigation of the molecular basis of phytoalexin production or disease resistance expression¹. However, the mechanisms by which such diverse elicitor molecules induce phytoalexin accumulation in plants are unknown. I have found⁴ that levels of glyceollin, a phytoalexin produced by soybean [*Glycine max* (L.) Merr.] hypocotyls in response to infection with the fungal pathogen *Phytophthora megasperma* var. *sojae* A. A. Hildb., are regulated by relative rates of induced biosynthesis and constitutive degrading activity. I report here the effects of various biotic and abiotic elicitors on biosynthesis and degradation of glyceollin in soybean tissues.

Detached cotyledons from 8-d-old seedlings of the soybean cultivar Harosoy 63 were wounded as described by Ayers *et al.*⁵, but wounds were made on the lower surface of the cotyledons. A 80 μ l drop of aqueous biotic or abiotic elicitor solution containing rifampicin and ampicillin at 10 and 500 μ g ml⁻¹, respectively, was placed on to the wounded areas of 10 cotyledons and these were incubated on moist filter papers in Petri dishes at 25 °C. Glyceollin did not accumulate in the control (H₂O-treated) cotyledons (Fig. 1a) despite the fact that significant levels of biosynthetic activity were present ~10 h after wounding (Fig. 1b). Failure of the control cotyledons to accumulate glyceollin seemed to be due to the presence of glyceollin degrading activity (Fig. 1c). Thus there was apparently a balance between synthesis and degradation, such that glyceollin did not accumulate. It would therefore be expected that glyceollin accumulation could be elicited by treatments that either inhibited its degradation and/or enhanced its synthesis to exceed the rate of degradation.

Treatment of the cotyledons with a biotic elicitor such as cell walls of *Phytophthora megasperma* var. *sojae*, a fungus highly pathogenic to certain cultivars of soybean¹, greatly stimulated the glyceollin biosynthetic activity of cotyledons (Fig. 1b) and resulted in considerable accumulation (Fig. 1a). The biotic elicitor, however, had no significant effect on glyceollin degrading activity (Fig. 1c). In contrast, an abiotic elicitor such as HgCl₂ also elicited glyceollin accumulation (Fig. 1a), but strongly inhibited glyceollin degrading activity of the cotyledons (Fig. 1c) with only slight effects on biosynthetic activity (Fig. 1b). These results show that the primary mechanisms of action of the biotic and abiotic elicitors are distinctly different.

All the effective abiotic elicitors of glyceollin accumulation tested, including various metals and detergents, strongly inhibited glyceollin degrading activity but their effects on synthetic activity were relatively small (Table 1). Some abiotic elicitors such as CuSO₄ and K₂Cr₂O₇ partially inhibited synthetic activity and glyceollin accumulation in response to these abiotic elicitors was reduced relative to those that did not inhibit synthetic activity (Table 1). High concentrations (10 mM) of some abiotic elicitors such as HgCl₂ and CdCl₂ did not induce glyceollin accumulation, possibly because of their high toxicity to plant cells at these concentrations as indicated by strong inhibitory effects on synthetic activity. Chemicals such as CoCl₂ and CrCl₃ that inhibited synthetic activity more strongly than degrading activity or those such as CsCl, SnCl₄ and low concentrations (0.1 mM) of HgCl₂ and CdCl₂, that had no significant effect on either synthetic or degrading activity were also inactive as elicitors of glyceollin accumulation. Thus, only those chemicals that selectively inhibited glyceollin degrading activity but not its synthetic activity were active abiotic elicitors.

In contrast to abiotic elicitors, none of the tested biotic elicitors of fungus origin inhibited glyceollin degrading activity, but all stimulated its synthetic activity to ~seven to eight times that in the control cotyledons (Table 1). Stimulation of the synthetic activity seemed to be caused only by biotic substances with elicitor activity, since soybean cell walls or any of the other biotic substances listed in Table 1 did not significantly affect glyceollin synthesis or accumulation. Thus the probable mechanism of action of biotic elicitors is stimulation of glyceollin synthesis to rates exceeding its rate of degradation.

Although the mechanisms underlying accumulation of phytoalexins by biotic elicitors are not understood¹, my earlier observations^{4,6-8} indicate that glyceollin accumulation in response to inoculation of soybeans with incompatible races of *Phytophthora megasperma* var. *sojae* may be due to increased biosynthesis resulting from *de novo* transcription of DNA. The present study indicates that such enhanced *de novo* biosynthesis may also occur in soybean after treatment with biotic elicitors, but not abiotic elicitors. The initial 'recognition' of fungal biotic elicitors by soybean cells is being studied in several laboratories^{1,5,9,10}.

The unique response of soybean cotyledons to abiotic elicitors is significant because it offers insight into the frequently observed accumulation of plant phytoalexins in response to 'stress', that is a change in the normal physiological state of the plant cell environment. The present data clearly indicate that phytoalexin accumulation can occur in response to mechanical wounding of plant tissue in conjunction with inhibition of the normal, apparently constitutive phytoalexin degrading system.

The lack of specificity of the phytoalexin response to various abiotic elicitors has been used as an argument against a role of phytoalexins in disease resistance¹. My results, however, suggest that this nonspecific phytoalexin response should be dis-

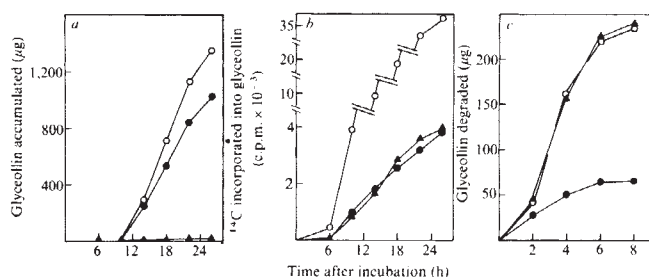


Fig. 1 Effects of ○, a biotic elicitor [Fungal cell wall (FCW), 50 μ g ml⁻¹] and ●, an abiotic elicitor [HgCl₂, 1.5 mM] on accumulation (a), synthesis (b) and degradation (c) of glyceollin in soybean cotyledons. For measurement of glyceollin accumulation, the wound droplets on the elicitor-treated cotyledons were collected by elution with water at various times after incubation, and glyceollin was extracted by ethylacetate⁵ and quantitated by a TLC/UV method⁶. For estimation of glyceollin biosynthetic activity in the elicitor-treated cotyledons, each wound droplet was replaced by 10 μ l of ¹⁴C-L-phenylalanine solution (15 μ Ci ml⁻¹, 522 mCi mmol⁻¹) at various times after incubation as indicated on the abscissa and the isotope was fed for subsequent 1 h. The wounded sides (~1 mm thick) of 10 cotyledons were then collected immediately and rates of ¹⁴C incorporation into glyceollin were determined by modifications of the TLC/UV method⁶ as described elsewhere⁴. For measurement of glyceollin degrading activity, eight freshly-collected cotyledons were sliced into halves parallel to their upper and lower surfaces and incubated in 50 ml flasks containing 10 ml of water, 300 μ g of purified glyceollin, antibiotics as above, and a biotic or abiotic elicitor at the indicated concentrations. The flasks were shaken at 60 strokes per min at 25 °C for various times and glyceollin remaining undegraded in the flasks was extracted by homogenisation with 20 ml of ethanol and quantitated⁶. FCW was prepared from *Phytophthora megasperma* var. *sojae* grown in a soybean hypocotyl medium⁶ for 5 d by the method of Ayers *et al.*⁹ but fungal homogenate was prepared by pulverising mycelium under liquid N₂ in a mortar and suspending in 0.5 M potassium phosphate (pH 7.2) followed by a brief sonication (1 min, 5 times). The concentration of the biotic elicitor is based on glucose equivalent by anthrone assay. ▲, Controls (water only).

Table 1 Effects of biotic and abiotic elicitors on accumulation, synthesis, and degradation of glyceollin in soybean cotyledons

Elicitor	Elicitor concentration	Accumulation (μg)	Glyceollin synthesis (% of control)	Degradation (% of control)
H ₂ O (control)	—	<20	100 (3,400–4,200 c.p.m.)*	100 (220–245 μg)*
<i>Effective abiotic elicitors</i>				
HgCl ₂	1.5 mM	972 $\pm$ 202	110 $\pm$ 34	28 $\pm$ 8
CdCl ₂	1.0 mM	932 $\pm$ 219	138	48
AgNO ₃	3.3 mM	1,280 $\pm$ 274	202 $\pm$ 56	19 $\pm$ 10
CuSO ₄	10.0 mM	472 $\pm$ 125	35	17
K ₂ Cr ₂ O ₇	3.3 mM	495 $\pm$ 194	42	29
Triton X-100	5.0 mg ml ⁻¹	1,391 $\pm$ 320	105 $\pm$ 10	28 $\pm$ 6
Nonidet P-40	5.0 mg ml ⁻¹	1,205 $\pm$ 295	98 $\pm$ 5	25
<i>Ineffective abiotic elicitors</i>				
HgCl ₂	0.1 mM	<20	119	92
	10.0 mM	<20	6	11
CdCl ₂	0.1 mM	<50	105	97
	10.0 mM	<100	9	28
CoCl ₂ †	10.0 mM	<100	28	55
CrCl ₃ †	10.0 mM	<20	18	48
CsCl†	10.0 mM	<30	139	97
SnCl ₄ †	10.0 mM	<20	113	104
<i>Effective biotic elicitors</i>				
Fungal extracellular metabolite	5.0 μg ml ⁻¹	1,352 $\pm$ 281	691 $\pm$ 181	102 $\pm$ 2
	20.0 μg ml ⁻¹	1,605 $\pm$ 309	831 $\pm$ 274	103 $\pm$ 5
Fungal cell wall	50.0 μg ml ⁻¹	1,295 $\pm$ 382	756 $\pm$ 126	97 $\pm$ 5
	250.0 μg ml ⁻¹	1,895 $\pm$ 225	856 $\pm$ 205	101 $\pm$ 4
Fungal cytoplasmic supernatant	100.0 μg ml ⁻¹	1,025 $\pm$ 125	721	95
	500.0 μg ml ⁻¹	1,434 $\pm$ 194	777	93
<i>Ineffective biotic elicitors</i>				
Fungal culture medium control†	5.0 μg ml ⁻¹	<20	132	97
Soybean cell wall†	200.0 μg ml ⁻¹	<20	115	105
Soybean cytoplasmic supernatant†	200.0 μg ml ⁻¹	<20	142	98

Amounts of glyceollin accumulated and degraded were measured at 24 and 8 h, respectively, and rates of synthesis at 18 h after incubation of cotyledons with elicitors, as described in Fig. 1. For preparation of fungal extracellular metabolite, *Phytophthora megasperma* var. *sojae* was cultured as described in Fig. 1. After fungal mycelium was removed by filtration, the culture fluids were lyophilised, dissolved in 10 mM Tris-HCl (pH 7.2), and passed through a column of Sephadex G-50 equilibrated with the same buffer. Fractions eluted in the column void volume were used. Fungal cytoplasmic supernatant was prepared by centrifuging a mycelial homogenate of the same fungus in 10 mM Tris-HCl (pH 7.2) at 80,000 g for 30 min and by dialysing the resulting supernatant against the same buffer. Fungal culture medium control was the fraction corresponding to the fungal extracellular metabolite fraction but the medium was not inoculated with the fungus. Soybean cell wall and cytoplasmic supernatant were prepared using the same methods described for preparation from the fungus. Means and standard errors are indicated where 3 to 5 replicate experiments were run, and other values are means of two replicate experiments.

* Actual values of incorporation of ¹⁴C-phenylalanine into glyceollin (c.p.m.) or of amounts of glyceollin degraded (μg) in the control cotyledons.

† Abiotic chemicals or biotic substances which did not induce glyceollin accumulation at all concentrations tested (0.033–20 mM for abiotic chemicals and 5–1,000 μg ml⁻¹ for biotic substances). Concentrations of effective abiotic elicitors were those approximately optimum in inducing glyceollin accumulation. Concentrations of all biotic substances were based on glucose equivalents measured by anthrone assay.

tinguished from the specific response associated with disease resistance, as it seems that the fungal metabolites operate differently from the abiotic elicitors.

The results presented here suggest that it may be possible to discover a new type of plant protectant, protecting against infection not through intrinsic antibiotic activity, but through an effect on plant-pathogen interaction resulting in higher accumulation of phytoalexins.

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The blind octopus, *Cirrothauma*

THE rare deep-sea octopod *Cirrothauma murrayi* Chun 1910 was first described from a single specimen caught during the Michael Sars Expedition of 1910 (ref. 1). Until now it has been caught only four more times². We describe here three specimens of this species that were recently caught during biological cruises of RRS Discovery (Fig. 1). All of these animals, including the Discovery ones, have been caught at depths of more than 1,500 m, except one that was dip-netted through the ice of the Arctic Ocean³.

Cirrothauma belongs to a poorly known family, the Cirroteuthidae, which have cirri along the arms, a pair of fins, a deep interbranchial web and no radula¹. *Cirrothauma* is reddish-brown semi-transparent and gelatinous in consistency (Fig. 1). Photographs of other cirroteuthids taken with deep-sea cameras have shown them swimming close to the bottom and looking like medusae, with head down and arms and web widely extended^{4,5}. In other photographs they are swimming backwards with a more typical cephalopod posture⁵.

The eyes are remarkable, being simple open cups, with no trace of lens, iris or ciliary body (Fig. 2). The cup is closed by a transparent continuation of the sclera and covered by a thin gelatinous layer less than 1 mm thick. The eyes were only 3 mm in diameter in Chun's animal, the mantle length being 40 mm. Our specimens are larger (155 mm (A), 130 mm (B) and 220 mm (C) mantle length) with an oval pupil 9 mm × 12 mm in the smallest (Fig. 2). The retinae are better developed than is implied from Chun's description¹ (Fig. 3a). However, the rhabdomes are not regularly packed as they are in *Octopus*, and there is no limiting membrane covering their outer ends, which therefore turn and taper irregularly. In one of the Discovery specimens part of the retina was folded, to make a series of chambers, lined by separate, single rhabdomes, looking, as Chun put it, "like little flames"¹ (Fig. 3b). The retina of specimen B therefore seems to be in a degenerating condition similar to that of some deep-sea fishes⁶.

The optic nerves are very long and pass through a large mass of tissue, the 'white body', to a minute optic lobe (Fig. 2). The structure of this is very simple, it lacks the peripheral layers of small cells that are characteristic of cephalopods with good vision. The vertical lobe and other parts of the visual memory system, so well-developed in *Octopus vulgaris*⁷, are reduced. It is difficult to understand what use may be made by the animal of a photoreceptor system such as this, with neither lens nor pin-hole aperture. That the animal has some use for photoreceptors is shown by the fact that the epistellar bodies, photosensitive vesicles near the stellate ganglion, are especially large in *Cirrothauma*.

There are large sac-like statocysts, suspended by muscles at some distance from the brain. As in other octopods there are inner and outer sacs separated by a perilymph (Fig. 4). The maculae are long oval patches with small circular statoliths not covering the whole area. There are no maculae neglectae. The

Fig. 1 *Cirrothauma murrayi* Chun 1910 photographed on board RRS Discovery while still just alive. The posterior end of the mantle is translucent and parts of the fins are transparent. Marks from the nets can be seen on the skin. One eye is visible at the side of the long, slender funnel. The arms are united by the web along most of their length. The stalked 'suckers' lie towards the tips of the arms. Specimen B caught at 3,000–3,500 m, mantle length 130 mm, funnel length 61 mm.

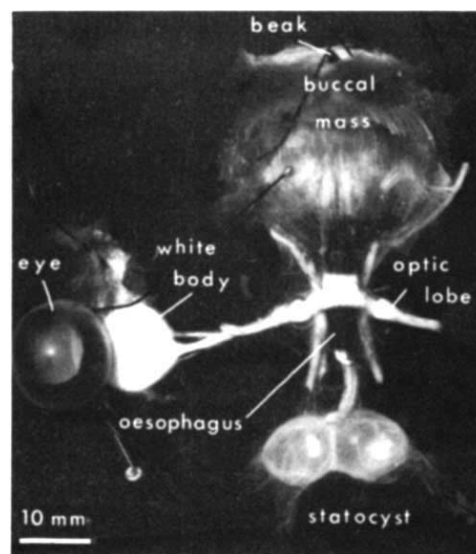
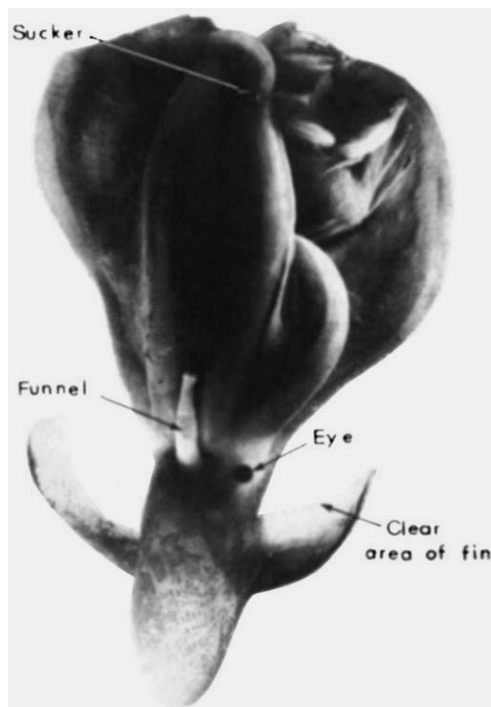


Fig. 2 The central nervous system, one eye, the statocysts and the buccal mass after dissection. The black rostral tip of the lower beak projects beyond the lips. The oesophagus is heavily pigmented and can be seen just behind the brain. Specimen B.

crista runs round the circumference of the sac, constricting it somewhat by a waist to form lateral and medial parts. There is a single anticrista, as in other octopods. The large volume of endolymph is presumably related to the need for the crista to detect slow angular rotations. This condition resembles that found in *Vampyroteuthis*, but the latter has several anticristae, as in decapods⁸.

The cirri are characteristic of the Cirroteuthidae and are absent from all other cephalopods with the exception of *Vampyroteuthis*. *Cirrothauma* has a longitudinal row of cirri on either side of the single row of suckers. Though not shown in Fig. 1, they can probably extend to a considerable length and are presumably tactile organs. They were stretched outwards and away from the arms in the cirroteuthids photographed in the sea⁵. Sections of the Discovery specimens show that the tactile centres of the brain are well-developed, unlike the visual system, and perhaps the cirri provide the main source of information about the surrounding conditions and for the detection of food. There are 38 suckers along each arm of our specimen of 155 mm mantle length (A) and 36 on the Chun (or 'type') specimen¹. Chun recognised two distinct sets of suckers, one being unique amongst cephalopods¹. He described the latter as 'tiny suckers situated on long, plump, spindle-shaped gelatinous stalks . . . 4–5 mm long in the middle of the arms and gradually become shorter towards the tips and proximally'. He suggested that they had lost their function as there was no opening. That they are suckers, even though they do not have an orifice, is shown in histological sections by the presence of a cuticle very similar to that found covering the infundibular surface of the suckers of *Octopus vulgaris* and other octopods⁹. In view of the presence of this sucker cuticle, it seems unlikely that Chun's¹ reserved interpretation that there were 'certain similarities with luminous organs' can now be upheld. Dr P. J. Herring (Institute of Oceanic Sciences) has also looked at the sections for us and can find no trace of light organs. The second type of sucker does have an opening and these suckers lie near the mouth, being the first six in both Chun's animal and in specimen A. The opening of the sucker is restricted and does not lead to a suction chamber such as is usually found in octopods⁹. There is a very shallow cup with radial muscles and a typical cuticle which, like that in other octopods, can be shed as a disk⁹.

The buccal mass is large as is the rostrum of the lower beak, which projects beyond the lips (Fig. 2). As in other cirroteuthids the radula is absent^{1,10}, in its place there is a fleshy tongue-like

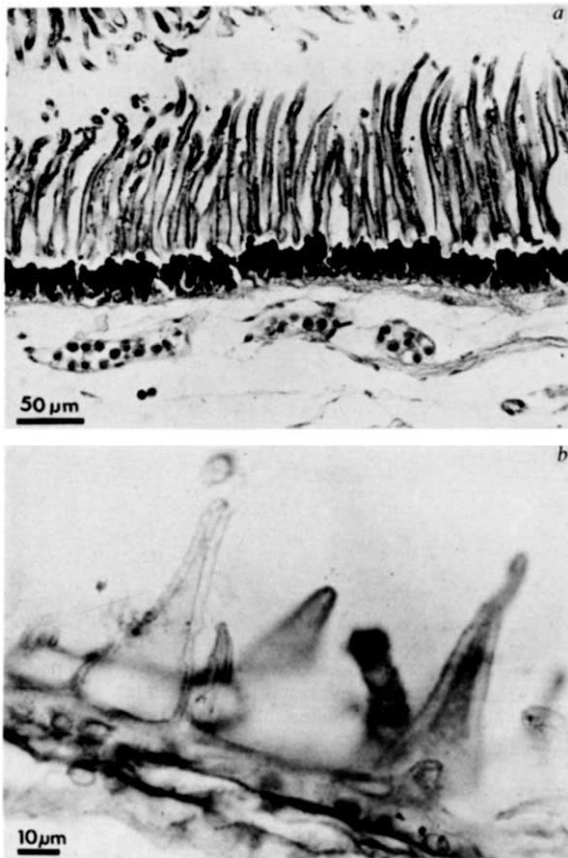
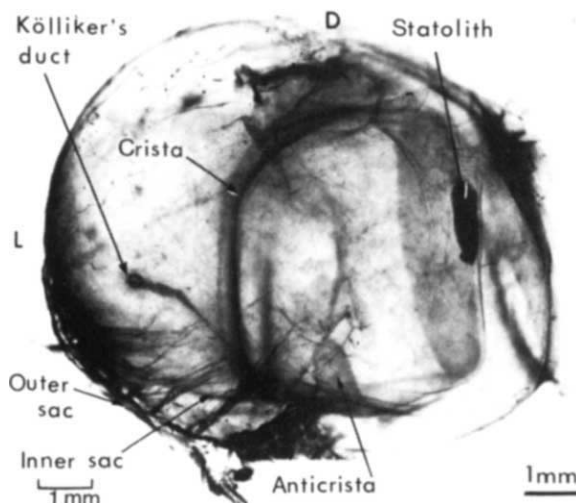


Fig. 3 a, Section of the retina of specimen C. b, Isolated rhabdoms from a sac formed by folding of the retina of specimen B.

structure attached ventrally along most of its length. At the front of this 'tongue' there is a large papilla, perhaps the salivary papilla.

The funnel is extremely long and slender¹, being 47, 61 and 185 mm in animals A, B and C, respectively (Fig. 1). The mantle opening is small and closely surrounds the base of the funnel. The function of this long funnel is not known but it could be important in directing fine jets of water during delicate manoeuvring movements while swimming upside down in the

Fig. 4 Left statocyst from specimen A seen from in front. The statolith is displaced and seen edge on near to the macula. D, dorsal; L, lateral.



medusoid form. Perhaps its jets of water are used to stir up the sediment on the seabed to expose small prey, or possibly to bury the eggs in soft sediment.

The cirroteuthids differ from all other octopods and resemble decapods in the presence of fins (Fig. 1). These, like the cirri, recall the condition in *Vampyroteuthis*¹¹. It seems probable that these deep-sea animals still retain characteristics common to both octopods and decapods. In this connection it is interesting that *Cirrothauma* and another cirroteuthid of which we also have sections, both have a system of giant nerve fibres¹¹, which are usually only associated with decapods¹². We hope that further study of the Discovery specimens may help to settle several problems of structure, function and mode of life of these curious animals.

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Spike frequency adaptation in amphibian sensory fibres is probably due to slow K channels

SPIKE frequency adaptation (SFA)—a decrease in firing frequency during the action of a maintained stimulus—is a mechanism for the input-output transformation of a signal and it is common at many levels of the animal kingdom. The occurrence of SFA in amphibian sensory nerves¹⁻⁴ offers an opportunity to explain the phenomenon at the membrane level in terms of the Hodgkin-Huxley (HH) theory⁵. Here we show that SFA in amphibian nerve is probably due to a current through slow K channels, and we suggest that these channels must also be responsible for different cases of SFA in nerve cells of various types.

The HH model for the node^{1,6} gives, for constant current steps, rhythmic firing but not SFA^{1,7}. Further mechanisms with relatively long time constants are obviously needed to account for SFA. As SFA lasts only from several ms to a few tens of ms it is evident that slow Na (ref. 8) and fast K (ref. 9) inactivations, with time constants of several hundred ms and even 1 s, cannot be a source for SFA. Electrogenic pumping is also excluded (for refs and discussion see ref. 10). K⁺ accumulation near the node¹¹ can be expected, by analogy with squid axon¹², not only to fail to cause SFA but also to give inappropriate changes of successive overshoot and undershoot amplitudes in a train, decreases rather than increases. Therefore, we consider that the necessary mechanism involves the currents flowing through slow K channels. The addition of slow K channels with the properties we have measured gives calculated SFA with most of the properties measured by others. Also, there are indications of slow K channels in amphibia in which SFA is usually observed, *Xenopus*

laevis^{2,4,13}, *Rana esculenta*^{3,4,14} and *R. ridibunda*^{4,15,16}. In the case of *R. ridibunda* many characteristics of the channels have been determined¹⁶, and they are used here for SFA calculations. We modified the HH model to present the overall K current (I_K) of the node as the sum of two components: a fast (I_{Kf}) and a slow (I_{Ks}) component.

The fast channel in *R. ridibunda* seems to behave like the familiar K channel in *R. pipiens*^{1,17}. The two channels were attributed to sensory nerve on the basis of their corresponding $n_{\infty} - E$ and $n_{\infty} - E$ curves on the E (membrane potential) axis, as had been done for *R. esculenta*¹⁸. I_{Kf} can thus be presented by the HH-Dodge model for *R. pipiens*¹. I_{Ks} can also be presented by an HH-type model, but differing in behaviour with E of the HH variable n_{II} describing the E -dependent gating kinetics of the slow K channel (ref. 16 and Fig. 1):

$$I_{Ks} = g_{Ks}(E - E_{Ks}) \quad (1)$$

$$g_{Ks} = \bar{g}_{Ks} n_{II}^4 \quad (2)$$

where g_{Ks} , $\bar{g}_{Ks}$ are, respectively, the slow K channel conductance and its maximum value; E_{Ks} is the K equilibrium potential for I_{Ks} .

In *R. ridibunda* the ratio $\bar{g}_{Ks}/\bar{g}_{Kf}$ is usually 0.2–0.8, depending on the frog specimen¹⁶. E_{Ks} is unknown. It was taken to be equal to E_{Kf} , that is, E_K in *R. pipiens* (–75 mV). The variable n_{II} can be represented fully by two functions: the steady-state relationship $n_{II\infty} - E$ and its kinetic characteristic $\tau_{nII} - E$. Both are given in Fig. 1.

For computations of spike trains, Hille's version of the HH-Dodge model¹⁷ was used. Small variations of maximum Na conductance with E (the term $(1 - E/183)$) were neglected so that we could follow quantitatively the critical firing level in terms of the theoretical relationship between firing potential and parameters entering the HH model¹⁹. To induce rhythmic activity the $h_{\infty} - E$ curve was shifted by 15 mV in the depolarising direction. Computations were carried out on a BESM-6 computer (Russian index) with a Fortran program.

Figure 2 shows the computed firing behaviour for the case when the contribution of I_{Ks} into the overall I_K was varied by variation of $\bar{g}_{Ks}$, that is, the density of the slow K channels. At $\bar{g}_{Ks} = 0$ the firing is purely rhythmic (Fig. 2a). However, SFA is well expressed even with $\bar{g}_{Ks}$ as small as 10% of $\bar{g}_{Kf}$ (Fig. 2b), and quickly increases with $\bar{g}_{Ks}$ (Fig. 2c,d) until damping of repetitive responses is complete (Fig. 2e).

Although Fig. 2 shows the simplest case, when an increase in I_{Ks} (a–e) is due to $\bar{g}_{Ks}$, it probably qualitatively covers most of the previously described basic features of SFA in amphibian nerve^{1–4}. One of them is a progressive rise in time of the overshoot and undershoot (for spikes following the first) amplitudes with nearly constant firing level. This is clearly seen in Fig. 2c and d. Further, the spike trains in amphibian nerves are finite, and this can be seen in Fig. 2d where, with sufficient density of slow K channels (that is, with sufficiently large $\bar{g}_{Ks}$), only one spike can be produced (Fig. 2e), which may be considered an extreme case of adaptation.

Thus, even the simplest model considered can account not only for SFA as such (a decrease in firing frequency with time) but also for accompanying phenomena (the character of changes of the overshoot and undershoot amplitudes and finite spike trains). Also any variation of parameters in equations (1) and (2) leading to greater I_{Ks} will lead to more pronounced SFA. This provides a variety of conditions for critical damping, as is the case in actual experiments (compare records of spike trains in refs 1–4). A proper choice of the starting value h_0 (refs 4, 7) and consideration of Na^+ accumulation near the inner surface of the nodal membrane¹⁰ and K^+ accumulation near its outer surface¹¹, would make the agreement between theory and experiment even better. There is, however, little hope of obtaining full details of SFA for a particular node, because this requires knowledge of the HH picture of all the currents involved and of the accompanying processes (such as Na^+ and K^+ accumulation, see above) on the same node. We aimed to show that the slow K channels are responsible for SFA in the node, and that this SFA

can be modified further by the various factors discussed here. The construction of models seems to be the best way to study basic features of SFA, so as to adjust relevant HH parameters and those of accompanying processes to fit experimental SFA or to look for interesting phenomena which can then be sought in an experiment. Our results indicate that slow K channels are responsible for SFA in amphibian nerve. In other amphibian

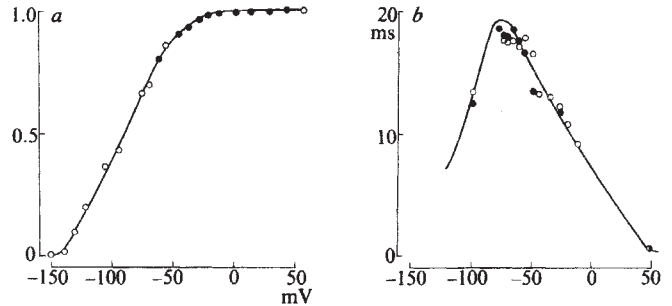


Fig. 1 a, Function $n_{II\infty}(E)$ for slow K channel used to compute the sensory nerve firing behaviour in Fig. 2. $\circ$, $n_{II\infty}$ values obtained by fitting $I_K - t$ curves associated with depolarising E steps from various values of E_1 (given by abscissa) to $E_2 = 50$ mV by searching with least squares the parameters of the equation for overall I_K :

$$I_K(t) = g_{Kf}(t)(E - E_{Kf}) + g_{Ks}(t)(E - E_{Ks})$$

where I and II refer to fast and slow K channels, respectively. I_{Ks} was measured in 128 mM KCl and 3×10^{-4} mM tetrodotoxin, with leakage current subtracted. As $E_{Kf} = E_{Ks}$, $n_{II\infty}(50 \text{ mV}) = n_{I\infty}(50 \text{ mV}) = 1$, and there is no K^+ accumulation near the node in this solution¹⁶, the equation acquires the HH form:

$$g_K(t) = \bar{g}_{Kf} \cdot \left[1 - (1 - n_{I\infty}(E_1)) \exp\left(-\frac{t}{\tau_{nI}}\right) \right]^{x_I} \cdot \bar{g}_{Ks} \cdot \left[1 - (1 - n_{II\infty}(E_1)) \exp\left(-\frac{t}{\tau_{nII}}\right) \right]^{x_{II}}$$

The terms: $\bar{g}_{Kf}$, $n_{I\infty}(E_1)$, τ_{nI} , x_I , $\bar{g}_{Ks}$ (where $\bar{g}_{Ks} = (1 - \bar{g}_{Kf})$), $n_{II\infty}(E_1)$, τ_{nII} , x_{II} in the latter equation were given values so that they fitted the whole family of experimental $I_K - t$ curves (now assumed to be the shape of the $g_K - t$ curves) associated with a set of E_1 . Parameters for channel I happened to be the same as for the K channel in *R. pipiens*^{1,17}. x_{II} was found to be 4 (as x_I), $n_{II\infty}(E_1)$ ($\circ$), τ_{nII} ($\bullet$) in b. Details of the fitting procedure are given elsewhere¹⁶. $\bullet$, $n_{II\infty}$ values obtained from the relationship $g_{Ks\infty} - E$ varying as $(g_{Ks\infty}/\bar{g}_{Ks})^{1/4}$. The relationship $g_{Ks\infty} - E$ was constructed from amplitudes of instantaneous I_K associated with repolarising E steps from various values of E_1 (given by abscissa) to the same value of E_2 , –100 mV (details are given in refs 15, 16). The $n_{II\infty} - E$ curve was drawn by eye and its ordinates were fed into the computer in 5-mV steps along the abscissa. b, Function $\tau_{nII}(E)$ for the slow K channel used for computation of sensory nerve firing behaviour in Fig. 2. τ_{nII} values were calculated from experimental time constants τ_{Ks} determining the decline of g_{Ks} tails following repolarisation from constant value of E_1 , 67 mV, to various values of E_2 (given by abscissa) by the following equation:

$$\bar{g}_{Ks} \cdot \left[(n_{II\infty}(E_1) - n_{II\infty}(E_2)) \exp\left(-\frac{t}{\tau_{nII}}\right) + n_{II\infty}(E_2) \right]^4 = (g_{Ks0} - g_{Ks\infty}) \exp\left(-\frac{t}{\tau_{Ks}}\right) + g_{Ks\infty}$$

where g_{Ks0} is the g_{Ks} value at the moment just after a repolarising step, and $g_{Ks\infty}$ is its steady-state value at E_2 . The solution was obtained for $t = \tau_{Ks}$ assuming $n_{II\infty}(67 \text{ mV}) = 1$ (see a), $g_{Ks0} = \bar{g}_{Ks} \cdot n_{II\infty}^4(67 \text{ mV}) = \bar{g}_{Ks} \cdot n_{II\infty}^4(E_2)$. For $n_{II\infty}$ of ~0.3 (that is, for $E_2 \geq -110$ mV), $\tau_{nII} \approx \tau_{Ks}/n_{II\infty}(E_2)$. τ_{nII} were scaled to 22°C (as in Hille's equations for τ_n in *R. pipiens*¹⁷) with a Q_{10} of 3.0. $\circ$, τ_{nII} measured, as described, with E_1 40 mV; $\bullet$, the same, with E_1 500 mV; $\bullet$, τ_{nII} obtained from depolarising E steps as described in a. The smooth curve was drawn by eye and its ordinates were fed into the computer in 5-mV steps along the abscissa.

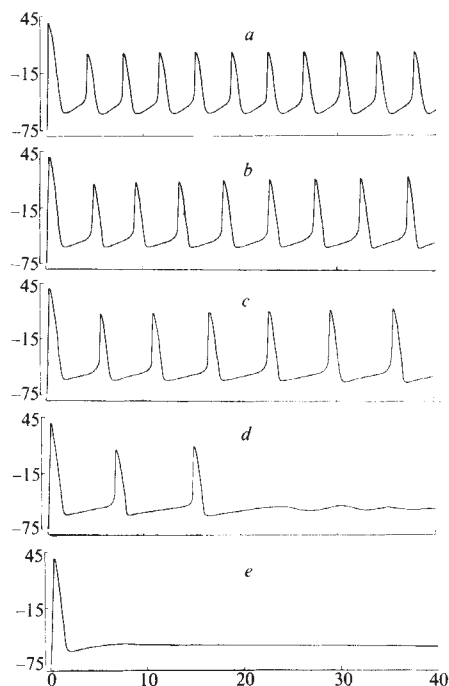


Fig. 2. Effect of the slow K current I_{KII} on firing behaviour of amphibian sensory nerve associated with a constant current step of 0.9 nA. Increase in I_{KII} is due to increase in $\bar{g}_{KII}$ (0, 10, 20, 30 and 40%, respectively, in a, b, c, d and e of $\bar{g}_{KII}$ (maximum K conductance through the fast K channels)). As I_{KII} in *R. ridibunda* is an additional process to the *R. pipiens* sensory nerve¹⁶, all the basic data necessary for this computation were those tabulated for *R. pipiens* by Hille¹⁷, except for h_0 which was chosen to be 0.9286 to induce the repetitive activity as in a. Abscissa: time (ms), ordinate: membrane potential (mV).

sensory nerves capable of SFA, the slow K channels are likely to be generally similar to those in *R. ridibunda*¹³, so that the physiological basis for SFA must be common to different amphibia.

Slow K channels might also provide a mechanism additional to or underlying that described¹⁸ for sensory compared with motor fibres. Sensory fibres respond to a constant current step by producing a train with increasing interspike intervals, whereas motor fibres respond by producing one or very few spikes³. Thus, motor fibres can be considered quickly adapting. The n_∞ -E curve for motor fibres was found to be displaced from that for sensory fibres in a hyperpolarising direction¹⁸, which damps repetitiveness (our unpublished calculations). However, this displacement and consequently the mechanism for sensory compared with motor fibres may be the result of greater $\bar{g}_{KII}$ in motor than sensory fibres, for $\bar{g}_{KII}$ is activated by more negative ranges of membrane potential than is $\bar{g}_{KI}$ (refs 15, 16). Thus, in Fig. 1 the 0.5 $n_{II\infty}$ value is at $E = -92$ mV whereas the 0.5 $n_{I\infty}$ value for *R. ridibunda* usually lies between -50 and -60 mV¹⁶, as 0.5 n_∞ for *R. pipiens*^{1,17}. Then the shift of the curve of n_∞ -E for overall $\bar{g}_K$ (as measured before¹⁸) will increase with $\bar{g}_{KII}$, that is, with the density of the slow K channels. It is tempting to suggest that neurones in the ventral roots synthesise more slow K channels than neurones in the dorsal roots.

The sensory nerves of *R. pipiens* usually seem to have only fast K channels. This follows from I_K measurements, indicating that they obey¹ the Cole-Moore translation principle²⁰, and from noise measurements suggesting a two-state open-closed transition for the K channels involved²¹. Both tests would fail if I_K in this node were due to more than one population of K channels (it was the analysis of I_K currents associated with the Cole-Moore translation test that led to the discovery of the slow K channels in *R. ridibunda*^{15,16}). However, as SFA also occurs (rarely) in *R.*

*pipiens*¹, our hypothesis suggests that a slow K channel must occur, if rarely, in this specimen as well, but that the yield of the gene product for the slow K channel is lower here than in other species.

The only other example for which SFA has been explained in terms of the HH theory concerns neurones from the mollusc *Archidoris*²². The SFA phenomenology here differs considerably from that in amphibian nerves, especially in that spike trains here are finite²². However, this is the picture that results from computation of the firing behaviour when the current through the slow K channel involved (which considerably differs from that in the node) is taken into account. Thus, in two markedly different cases of SFA (in the node and the neurone), the current through the corresponding slow K channel is sufficient to describe basic characteristic features of SFA. There are many different types of slow K channels in biological membranes (reviewed in ref. 23) and, except for (or together with) the cases where an electrogenic pump is involved²⁴, the diversity in SFA phenomenology in nerve cells of different types is probably mainly due to diversity of slow K channels. The mechanism discussed here might also apply to adaptational behaviour of sustained and transient receptors responding to a stationary component of generator potential, as shown in Fig. 2b, c and d, e, respectively.

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Labelling of benzodiazepine receptors *in vivo*

The presence of benzodiazepine receptors has been demonstrated using isolated membrane preparations from the mammalian central nervous system¹⁻³. Specific binding of ³H-diazepam to synaptosomal membranes is maximal at temperatures of 0-4 °C, but reduced by more than 95% at physiological temperatures⁴. Although the inhibition of ³H-diazepam binding by other benzodiazepines *in vitro* closely parallels their pharmacological potencies *in vivo*, failure to demonstrate specific binding in physiological conditions raises questions as to whether receptor binding occurs *in vivo*. We report here that benzodiazepine receptors can be demonstrated in the intact

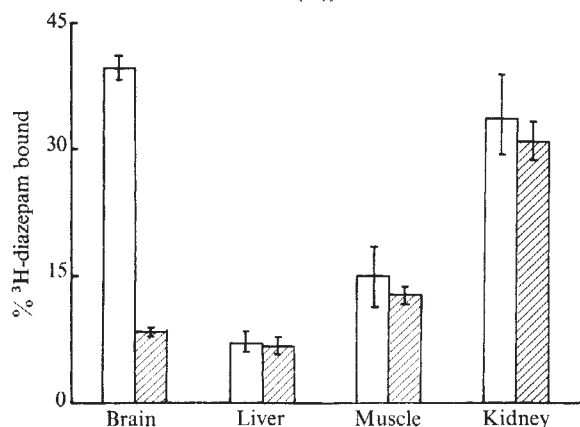
animal following intravenous administration of ^3H -diazepam. The labelling of benzodiazepine receptors *in vivo* permits studies of receptor occupancy in various pharmacological and physiological conditions as well as autoradiographic localisation of the receptor *in situ*.

Adult male Sprague-Dawley rats (125–175 g) were used in all studies. ^3H -Diazepam ($39.08 \text{ Ci mmol}^{-1}$, NEN) was diluted with 50% ethanol to a final volume of $300 \mu\text{l}$ and administered through the lateral tail vein. Studies in this (unpublished) and other laboratories⁵ have demonstrated that the maximum brain concentration of diazepam occurs within 1–2 min of intravenous administration. At this time 95% of the radioactivity in brain is authentic diazepam, as demonstrated by thin layer chromatography⁵. Animals were decapitated 1 min after injection and the tissues to be assayed were immediately dissected, weighed and frozen on dry ice (-70°C). Studies designed to test the effects of freezing on the amount of specifically bound ^3H -diazepam revealed no differences between fresh and frozen samples. Therefore, subsequent studies were carried out using frozen specimens, giving greater efficiency and control over assay and homogenisation time.

For assay of membrane-bound ^3H -diazepam, tissues were homogenised in 25 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.4) using a Brinkmann polytron (20 s, full speed). Aliquots of the homogenate were sampled for total and membrane-bound ^3H -diazepam. The latter was determined by filtering the homogenate (0.1–1.0 ml) through Whatman glass fibre filters (GF/B) followed by two rinses of ice-cold Tris-HCl buffer (5 ml each). The filters were then placed in scintillation vials containing 10 ml of Aquasol (NEN), shaken thoroughly and counted with a Beckman LS-250 liquid scintillation counter.

Membrane-bound radioactivity was linear with tissue concentration in homogenates containing up to 20 mg of brain tissue (wet weight). Preliminary studies were carried out to determine whether the specific membrane-bound radioactivity was a result of *in vitro* binding that might have occurred artefactually following homogenisation. In an experiment comparing the amount of specifically bound ^3H -diazepam with post-homogenisation incubation time, both specific and nonspecific binding were maximal at the earliest time tested (30 s post-homogenisation) and did not change over a 60-min period. As saturation of benzodiazepine receptors *in vitro* occurs approximately 10 min after incubation^{4,6}, these data support the assumption that the binding observed following intravenous administration had occurred *in vivo*. The results also demonstrate the stability of the drug-receptor complex when the preparation is kept at $0-4^\circ\text{C}$.

Fig. 1 Stereospecific inhibition of ^3H -diazepam binding *in vivo*. Rats were injected with $50 \mu\text{Ci } ^3\text{H}$ -diazepam in combination with the enantiomers of B9 or B10 ($700 \mu\text{g kg}^{-1}$). Values represent $\bar{X} \pm \text{s.e.m.}$ in brain ($n=8$), liver ($n=6$) and other tissues ($n=2$). Per cent of ^3H -diazepam bound is the amount of ^3H -diazepam in the homogenate retained by the GF/B filter divided by the amount of ^3H -diazepam in an equal volume of homogenate $\times 100$. ■, Active isomers (B9(+), B10(+)); □, inactive isomers (B9(-), B10(-)).



Co-injection of the ^3H -diazepam with unlabelled clonazepam ($250 \mu\text{g kg}^{-1}$) or the pharmacologically active benzodiazepine enantiomers B9(+) and B10(+) ($700 \mu\text{g kg}^{-1}$) dramatically reduced membrane-bound ^3H -diazepam ($40.7 \pm 2.1\%$ ($n=5$) compared with $7.72 \pm 1.54\%$ ($n=5$) bound) whereas the total concentration of ^3H -diazepam in brain was unaffected. Substitution of the pharmacologically inactive enantiomers B9(-) or B10(-) did not produce any significant decrease of membrane-bound ^3H -diazepam ($39.8 \pm 1.2\%$ bound). Homogenisation of the tissue in buffer (50 mM Tris-HCl, pH 7.4) containing $3 \mu\text{M}$ unlabelled diazepam resulted in a rapid displacement of more than 90% of membrane-bound radioactivity. The latter experiment agrees well with observations *in vitro* indicating that more than 90% of the ^3H -diazepam bound to crude synaptosomal fractions of brain is displaced by this concentration of diazepam^{1,3}.

Table 1 Regional distribution of benzodiazepine receptors *in vivo*

Region	Specific ^3H -diazepam binding (pmol per g tissue)	Nonspecific binding (% of total binding)
Cortex	12 ± 2.0	12
Hypothalamus	8.7 ± 0.7	19
Midbrain	7.6 ± 0.7	20
Striatum	6.8 ± 1.7	32
Septal region	6.2 ± 0.2	17
Cerebellum	6.2 ± 0.7	50
Pons medulla	5.5 ± 0.6	20

Rats were injected intravenously with $50 \mu\text{Ci } ^3\text{H}$ -diazepam. The various brain regions were hemisected, one half of each region being homogenised in 50 mM Tris buffer (pH 7.4) with or without $3 \mu\text{M}$ diazepam, and assayed in triplicate within 15 min of homogenisation. Specific binding is defined as the amount of ^3H -diazepam bound after homogenisation in Tris buffer minus the amount bound after homogenisation with $3 \mu\text{M}$ diazepam. Values represent the mean $\pm \text{s.e.m.}$ of brain regions from three rats.

As *in vitro* studies have shown that benzodiazepine receptors are highly localised to brain¹⁻⁴, it was of interest to study the tissue distribution of ^3H -diazepam binding *in vivo*. Figure 1 shows the binding of ^3H -diazepam in brain and peripheral tissues with and without co-injection of a pharmacologically active benzodiazepine. Although ^3H -diazepam binds to membranes of all the peripheral tissues studied, only the binding in brain is displaced in a stereospecific fashion. Similar results have been reported *in vitro*⁴. Finally, we investigated the regional distribution of benzodiazepine receptors in brain (Table 1). The rank order concentration of benzodiazepine receptors in various cortical and subcortical areas is identical to that observed in the *in vitro* assay⁴.

Our results demonstrate the feasibility of labelling benzodiazepine receptors *in vivo* in physiological conditions. These receptors are highly localised to the CNS, and to cortical areas in particular. Displacement of specific binding occurs only with pharmacologically active benzodiazepines, thus supporting a role for these sites as pharmacological receptors mediating the action(s) of these drugs within the CNS.

In addition to establishing specific binding of ^3H -diazepam to benzodiazepine receptors in physiological conditions, *in vivo* labelling of benzodiazepine receptors may also permit further studies on receptor changes during various pharmacological and/or physiological states, as well as autoradiographic visualisation of the receptor. Similar *in vivo* studies using ^3H -flunitrazepam have also been carried out (R. Chang and S. Snyder, personal communication).

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6896) and B10(–) (Ro 11-6893). S.M.P. and M.J.W. thank Dr J. Axelrod for providing laboratory facilities. P.S. thanks Dr. J. Daly and the NIAMDD for providing facilities.

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An endogenous protein modulates the affinity of GABA and benzodiazepine receptors in rat brain

BIOCHEMICAL^{1,2} and neurophysiological^{3–5} evidence has suggested that benzodiazepines may relieve anxiety facilitating the synaptic action of γ -aminobutyric acid (GABA), an important neurotransmitter in the brain of mammals, including man. As benzodiazepines fail to increase the turnover of GABA stored presynaptically as would be expected if they were to act as indirect GABA agonists, and since it has been difficult to demonstrate a direct agonistic action of the benzodiazepines on GABA postsynaptic receptors^{5–7} *in vitro*, the molecular mechanism whereby benzodiazepines facilitate GABAergic transmission is still unknown. Independent investigators^{8–10} have recently reported the presence of a high affinity, saturable, stereospecific binding site for benzodiazepines in synaptic membrane preparations obtained from brain of different animal species, including man. This high affinity binding is now used to determine the therapeutic potency and to study the mode of action of benzodiazepines in anxiety.

Any known transmitter, including GABA, fails to compete with the benzodiazepines for their specific brain receptors; however, the brain distribution pattern of these benzodiazepine receptors is similar to that of the Na^+ -independent high affinity binding sites for GABA (8,9). This and analogies in the pharmacological profile of GABA-mimetics and benzodiazepines^{2,11} has suggested to us that studies of the mechanism whereby benzodiazepine receptors interact with the Na^+ -independent GABA receptor sites may help to understand the mode of action of these antianxiety drugs.

The discovery that benzodiazepine receptors are present in the central nervous system has also led to the proposal¹² that: "by analogy with the discovery of opiate receptor, the benzodiazepine receptor assay may even offer a simple means for the discovery of a naturally occurring ligand for such receptor: the brain's own antianxiety substance".

Recently, we reported that a small thermostable protein ('endogenous inhibitor') which noncompetitively reduces the affinity of GABA for its Na^+ -independent recognition site, could be extracted from brain preparations enriched with synaptic membranes¹³. This endogenous inhibitor is thermostable (at 95°C for 10 min), has acidic characteristics, and when

purified 1,500-fold by gel filtration, ion-exchange chromatography, and preparative polyacrylamide gel electrophoresis, gives on 10, 15 and 20% SDS polyacrylamide gel only one major protein band equivalent to a molecular weight of ~15,000. The present report shows that pharmacologically-active benzodiazepines modulate the affinity of GABA for its recognition

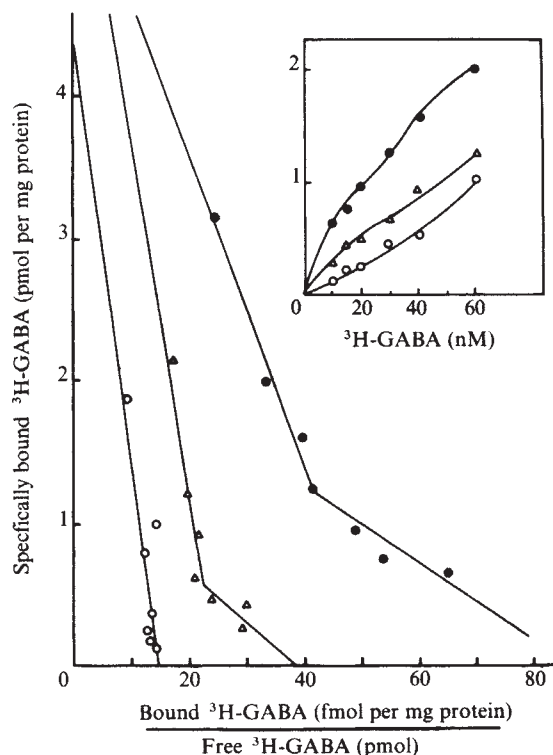


Fig. 1 Scatchard analysis of interaction between diazepam and 'endogenous inhibitor' of Na^+ -independent ^3H -GABA binding. Crude synaptic membranes from rat cerebral cortex¹⁵ were frozen at -20°C for 24 h and treated for 30 min at 37°C with 0.01% Triton X-100. After centrifugation at 40,000g for 10 min the pellet was dispersed with a polytron in 50 volumes of 50 mM Tris-citrate (pH 7.1) without Triton X-100. This operation was performed twice. Specific GABA binding was calculated by subtracting from the binding which occurred by incubating the membranes at 4°C for 5 min with ^3H -GABA, the binding that occurred by incubating with ^3H -GABA plus 10^{-3} M unlabelled GABA. Only ^3H -GABA (40 Ci mmol⁻¹) was used when the lowest concentrations of ligand (1–30 nM) were tested, and a constant amount of ^3H -GABA (30 nM) plus various concentrations (30–120 nM) of nonradioactive GABA when the highest concentrations of the ligand were tested. The ligand tested was contained in 100 μl which was incubated with a standard aliquot (200 μg protein) of membranes dispersed in 900 μl of 50 mM Tris-citrate (pH 7.1). After incubation the reaction was terminated by centrifuging at 48,000g for 10 min. The supernatant was discarded and the pellet rapidly rinsed with cold H_2O and suspended in NCS (Amersham-Searle solubiliser) before counting. To study the action of the endogenous inhibitor, membranes were incubated at 0°C for 15 min, with 7.5 μg of 500-fold purified endogenous inhibitor before the addition of ^3H -GABA. When the effect of diazepam was studied membranes were preincubated at 0°C for 15 min with 8×10^{-7} M diazepam before addition of inhibitor. Membranes incubated at 0°C without the inhibitor or diazepam served as controls. Scatchard analysis revealed two affinity components for GABA binding. The apparent K_D for the low affinity component was: 111; 240 and 286 nM for control, diazepam + endogenous inhibitor and endogenous inhibitor, respectively. The apparent K_D for the high affinity component was: 28 and 33 nM for control and diazepam + endogenous inhibitor, respectively. A high affinity component for ^3H -GABA binding was not measurable in membranes preincubated with the endogenous inhibitor alone. In the insert the values of the specifically bound (SB) ^3H -GABA are plotted against the concentrations of ^3H -GABA in the incubation mixture. Proteins were determined with the method of Lowry¹⁸. ●, Control; △, diazepam + inhibitor; ○, inhibitor only.

site by competing with the endogenous inhibitor. In addition, this endogenous protein reduces the apparent affinity of diazepam for its recognition site. To study whether diazepam could change the activity of the endogenous inhibitor, the inhibitor was purified 500-fold using a previously described procedure¹³. In brief, the entire rat brain was homogenised in 5 mM Tris-citrate buffer (pH 7.1). After centrifugation, the supernatant was incubated at 95°C for 10 min and successively dialysed filtered on CF 50A Amicon membrane and chromatographed on Sephadex G-100 column. It was then purified further with Dowex 50X8 ion-exchange chromatography where it behaved as an acidic protein.

As shown in Fig. 1a, the addition of this purified inhibitor to a crude preparation of synaptic membranes free of endogenous inhibitor (the inhibitor was removed by freezing and thawing and by treatment with Triton X-100, for details see Fig. 1 legend), resulted in a substantial change of the kinetic characteristics of Na⁺-independent ³H-GABA binding. Scatchard plot analysis of these data indicates that the addition of purified endogenous inhibitor to crude synaptic membrane preparations concealed the Na⁺-independent high affinity recognition sites for GABA and also changed the kinetic characteristics of the low affinity GABA binding sites (see Fig. 1). If the membranes were preincubated with diazepam, the action on the endogenous inhibitor on both high and low affinity components of GABA binding was reduced (Fig. 1). The data of Fig. 1 indicate that the action of the endogenous inhibitor on GABA binding is apparently noncompetitive, and suggest a negative cooperative interaction between the inhibitor and the GABA recognition sites. Diazepam added in concentrations up to 10⁻⁵ M failed to change the binding of ³H-GABA to an inhibitor-free crude synaptic membrane preparation, but increased the affinity binding of ³H-GABA to a fresh crude synaptic membrane preparation (see also ref. 14). Freshly prepared membranes revealed Na⁺-independent binding for ³H-GABA with an apparent *K*_D of ~200 nM (low affinity component)¹⁵. As we have previously reported¹³, the appearance of a high affinity component for ³H-GABA binding in the fresh membrane preparation is prevented by the presence of the endogenous inhibitor. When fresh membranes were preincubated with diazepam (5 × 10⁻⁷ M) there was a change in the kinetic characteristics of the ³H-GABA binding due to the appearance of a high affinity site with an apparent *K*_D of 36 nM in addition to the low affinity binding component (*K*_D = 200 nM). Hence, the addition of diazepam to a fresh membrane preparation changes the kinetics of GABA binding in a way similar to that elicited by the removal of the endogenous inhibitor by freezing, thawing washing with Tris buffer and treatment with Triton X-100 as described previously¹³.

The nature of the interaction between the various benzodiazepines and the endogenous inhibitor was then studied by challenging different amounts of the endogenous inhibitor with a constant concentration of benzodiazepines. Figure 2a and b show the effects of 10⁻⁶, 10⁻⁷ and 10⁻⁸ M diazepam and of 10⁻⁷ M Ro-11(+) (a pharmacologically active benzodiazepine, see Fig. 2 legend) and Ro-11(-) (the inactive isomer) on the inhibition of the high affinity ³H-GABA binding induced by the addition of increasing amounts of the endogenous inhibitor to the inhibitor-free membranes. In the presence of benzodiazepines, the amount of inhibitor required to produce an inhibition of the high affinity GABA binding was increased. The difference was particularly significant when small doses of the inhibitor were used. For example, 2.5 µg of the endogenous inhibitor produced ~50% inhibition of the GABA binding in controls or in Ro-11(-)-treated membranes. The same amount of the endogenous inhibitor produced 10, 18 and 32% inhibition in the presence of 10⁻⁶, 10⁻⁷, 10⁻⁸ M diazepam, respectively and 12.5% in the presence of 10⁻⁷ M Ro-11(+). The double reciprocal plot of the data indicates an apparent competitive interaction between the purified endogenous inhibitor and diazepam or Ro-11(+) (see inserts of Fig. 2a and b). The Hill plot analysis of the data gives a Hill coefficient for diazepam- and

Ro-11(+)-treated membranes higher than the Hill coefficient for membranes treated with solvent or Ro-11(-). This confirms that the negative cooperative action of the endogenous inhibitor on ³H-GABA binding is reverted by pretreating the membranes with pharmacologically active benzodiazepines.

We have also tested the potency of a few benzodiazepines to compete with the endogenous inhibitor of GABA binding. Flunitrazepam and clonazepam were four to five times more potent than diazepam but diazepam was at least 10 times more potent than chlordiazepoxide and medazepam. These results suggest that the potency of different benzodiazepines in preventing the inhibition of the high affinity Na⁺-independent GABA binding by the endogenous inhibitor correlates with their binding affinity to saturable sites in rat brain membrane preparations^{8,9} and with the known pharmacological *in vivo* potency as antianxiety agents¹⁶.

The interaction between benzodiazepines and the endogenous inhibitor of Na⁺-independent GABA binding raises the question of whether the endogenous inhibitor interacts with benzodiazepines because it functions as a soluble benzo-

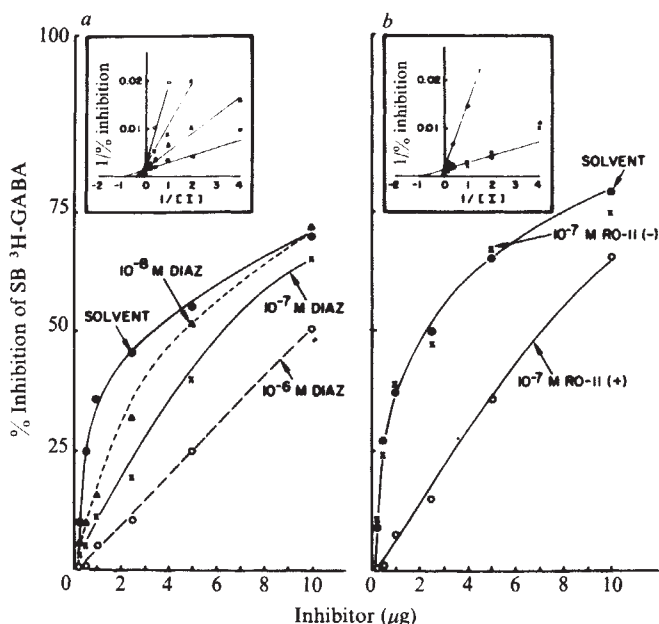


Fig. 2 Interaction of diazepam (a) or Ro-11 (b) with various amounts of endogenous inhibitor of Na⁺-independent ³H-GABA binding. Crude synaptic membranes from rat cerebral cortex were prepared by successive freezing, thawing and washing with Tris buffer plus treatment with Triton X-100 as for Fig. 1. Membranes were preincubated at 0°C for 15 min with diazepam (a) or Ro-11(+) or Ro-11(-) (b) before addition of inhibitor. In the insert double reciprocal plot of the data is shown, revealing an apparent competitive interaction between diazepam, Ro-11(+) and the endogenous inhibitor (I). This gives *K* values of 0.91 µg of inhibitor for controls, 3.3, 5.2 and 10.2 µg for 10⁻⁸ M, 10⁻⁷ M and 10⁻⁶ M diazepam, respectively, and 9.2 µg of inhibitor protein for 10⁻⁷ M Ro-11(+). Ro-11(+)=Ro-113128; [(+)-5-(*O*-chlorophenyl)-1,3-dihydro-3-methyl-7-nitro-2*H*-1,4-benzodiazepine-2-one] is pharmacologically active and Ro-11(-)=Ro-113624 is inactive. Results similar to those reported here were obtained in five experiments with diazepam and in five with Ro-11. The average dose of the inhibitor which produced a 25% inhibition of the GABA binding is 0.5 ± 0.018 µg in 10 preparations of the control membranes, 25% inhibition was achieved with 1.5 µg (±0.082), 2.5 µg (±0.10) and 4 µg (±0.28) of endogenous inhibitor in the presence of 10⁻⁸, 10⁻⁷ and 10⁻⁶ M of diazepam, respectively. A 25% inhibition was achieved with 0.45 µg (±0.020) of endogenous inhibitor in the presence of Ro-11(-) and with 3.8 µg (±0.30) in the presence of Ro-11(+). The difference between the controls and the Ro-11(-) is not significant. The difference between the controls and different diazepam doses among the different benzodiazepines doses and between Ro-11(-) and Ro-11(+) is statistically significant (*P* < 0.05).

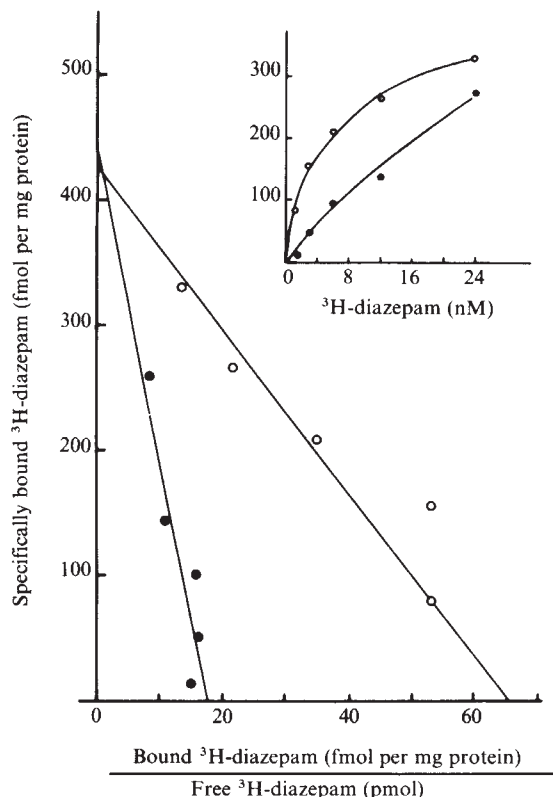


Fig. 3 Scatchard plot of the ^3H -diazepam binding to control or endogenous inhibitor-treated crude synaptic membranes. Frozen, thawed and Triton X-100 treated crude synaptic membranes were prepared as described in Fig. 1. Specific ^3H -diazepam binding was calculated by subtracting from the binding which occurred by incubating the membranes at 4°C for 15 min with ^3H -diazepam ($0.75\text{--}24\text{ nM}$; 40 Ci mmol^{-1}), the binding that occurred by incubating with ^3H -diazepam and 10^{-6} M unlabelled diazepam⁹. Concentrations ($0.75\text{--}24\text{ nM}$) of ^3H -diazepam ($100\text{ }\mu\text{l}$) were incubated with a standard aliquot ($200\text{ }\mu\text{g}$ protein) of membranes dispersed in $900\text{ }\mu\text{l}$ of 50 mM Tris-citrate ($\text{pH } 7.1$). The equilibrium binding was terminated by filtration under vacuum through Whatman CF/B glass-fibre filters which were washed twice with 5 ml ice-cold Tris-citrate buffer $\text{pH } 7.1$. When the action of the endogenous inhibitor was studied, the membranes were incubated at 0°C for 60 min in presence of $30\text{ }\mu\text{g}$ of 500-fold purified endogenous inhibitor before addition of ^3H -diazepam. Membranes incubated at 0°C for 60 min without diazepam served as controls. Scatchard analysis gave a K_D of $\sim 6\text{ nM}$ for controls and 24 nM for inhibitor-treated membranes. In the insert, the values of specifically bound (SB) ^3H -diazepam are plotted against the concentrations of ^3H -diazepam in the incubation medium. $\circ$, Controls; $\bullet$, inhibitor present.

diazepine binding site or as an endogenous modulator of the benzodiazepines for their recognition site in crude synaptic membranes. In fresh membranes (rich in endogenous inhibitor), the extent of diazepam binding is less than in frozen and Triton X-100 treated membranes (free of endogenous inhibitor). Thus, these results exclude the possibility that the endogenous inhibitor acts as a soluble benzodiazepine recognition site.

The data reported in Fig. 3 indicate that the endogenous inhibitor has some of the characteristics expected of a molecule that modulates the affinity of benzodiazepine receptors for this drug. The affinity of ^3H -diazepam for frozen Triton X-100-treated membrane preparations preincubated at 0°C for 60 min with the endogenous inhibitor was reduced when compared to that of similar membranes preincubated without endogenous inhibitor (Fig. 3). The action of the endogenous inhibitor on ^3H -diazepam binding (see Fig. 3) at 0°C was evident only when the endogenous inhibitor was preincubated with the membranes for 15 min or more. Preincubation of crude synaptic membranes with the endogenous inhibitor failed to change the kinetic profile of the recognition site of muscarinic receptors as studied by QNB binding¹⁷.

In summary, the present experiments (compare Figs 1 and 3) indicate that an endogenous inhibitor protein isolated and purified from fresh membrane preparations acts noncompetitively with GABA for its Na^+ -independent recognition site and interacts with diazepam binding. The function of diazepam binding is expressed as a modulation of the affinity of GABA recognition sites. Although binding of diazepam or of endogenous inhibitor modifies GABA binding, they do not exert this effect by binding to GABA binding sites. This regulatory site may be linked to the high affinity site for benzodiazepine binding for which this endogenous inhibitor may act as a modulator or a natural agonist.

This inference agrees with the lack of competition between GABA and ^3H -diazepam for specific binding to brain membranes: moreover, it is in keeping with the relationship between the brain location of ^3H -diazepam and ^3H -GABA binding sites⁹ and is supported by the pharmacological interactions between diazepam and GABA receptor blockers^{1,3}. The apparent decrease in the affinity for ^3H -diazepam binding in brain synaptic membranes pretreated with the endogenous inhibitor could suggest that the endogenous inhibitor is functioning as a natural ligand for the benzodiazepine receptors. However, interpretation of these data requires caution because the time constants of our measures are not sufficiently short. In fact, we are dealing with a very complex system in which the theoretical assumption of a rapid reversible equilibrium implied by any inference derived from the Scatchard analysis may not be satisfied. Although our data clearly indicate that the 'endogenous inhibitor' may act as an 'endogenous modulator' of the benzodiazepine binding, it is at present impossible to establish whether this occurs as a result of a direct action at the high affinity receptor sites for the benzodiazepines or indirectly through an allosteric effect which involves the GABA/benzodiazepine/ Cl^- ionophore supramolecular complex. Studies are now in progress to elucidate this problem. These studies may also help to clarify the supramolecular nature of the postsynaptic GABA receptors and the role of the endogenous inhibitor protein in the regulation of the affinity of ^3H -GABA for the Na^+ -independent GABA recognition site. Preliminary work in our laboratory indicates that diazepam displaces the endogenous protein inhibitor from its physiological binding sites also *in vivo*. Whether a change in the amount of this endogenous inhibitor bound to synaptic membranes plays a part in determining the level of anxiety is not yet known but this can now be investigated.

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Is there a correction mechanism in the 5S multigene system?

RIBOSOMAL GENES are present in many copies per cell in all living species. They are the classic example of the tandemly repeated multigene system, and have been studied in a wide variety of organisms, including man^{1,2}, *Drosophila*^{3,4}, *Xenopus*^{5,6} and *Escherichia coli*⁷. Reiteration of these genes is presumably necessary to ensure the production of the large amounts of ribosomal and 5S RNA required for use in the ribosomes. However, the possession of multiple copies of a gene poses a special problem for evolution. If a lethal or deleterious mutation occurred in one gene of, for example, the 24,000 present for the 5S DNA in *Xenopus*⁵, this would have no selective disadvantage for the organism. What then prevents the accumulation of lethal mutations in such a system? Presumably if the number of effective genes were reduced significantly natural selection would operate. But it has been suggested that a 'correction mechanism'⁸ exists to maintain the homogeneity of the multigene system without a requirement for natural selection. We show here that two cloned repeats of *Xenopus* oocyte 5S DNA are very similar but not identical in sequence. The similarity strongly suggests that a correction mechanism is operating, but it is not so precise as to cause the different repeats to be identical. A small but distinct degree of heterogeneity is tolerated.

Two main kinds of correction mechanism have been proposed—'sudden' and 'gradual'. The master-slave hypothesis⁹ (a sudden mechanism) envisages the matching of a number of 'slave' sequences to a single 'master' once per life cycle. Thus neighbouring repeats or slaves should be identical. In the oocyte 5S DNA of *Xenopus laevis* adjacent repeating units are clearly heterogeneous in length¹⁰, ruling out this mechanism. A gradual mechanism called 'crossover fixation' has been proposed by Smith¹¹ and envisages a high rate of unequal crossing over, causing the fixation of multiple copies of a single ancestral repeat. This seems a more plausible theory, but the extent to which it operates is uncertain because the real extent of correction or homogeneity between the members of a multigene system has never been absolutely clear.

We have examined the extent of correction in detail for the oocyte 5S DNA gene system in *Xenopus laevis*. This DNA consists of a high A+T rich spacer made up of many short subrepeats followed by a G+C rich region which is a duplication of 174 nucleotides, containing the 5S gene and also a 'pseudogene' structure. The overall organisation of a repeat unit is shown in Fig. 1. Previous work on the oocyte 5S RNA¹²⁻¹⁴ has shown that it is a mixture of molecules, but the real extent of heterogeneity could not be assessed accurately¹³. Recently the entire sequence of total frog 5S DNA was determined^{15,16} but, again, microheterogeneity remained undetected, because a mixture of sequences was studied. We have compared the entire sequence of two cloned repeat units of oocyte 5S DNA, Xlo 5

and Xlo 31. These are by definition unique species, so that the sequences obtained must actually exist and are not averaged¹⁷.

The sequence of these repeat units was determined by the Maxam and Gilbert technique¹⁸. Typical sequencing gels are shown in Fig. 2. The complete sequence of clone Xlo 5 is shown in Fig. 3 with the differences present in clone Xlo 31 indicated. Two types of microheterogeneity were found. The first involved the addition or deletion of oligonucleotide blocks in the A+T rich spacer region. This phenomenon has been previously noted

Fig. 2 Two thin gels¹⁹ showing different parts of the sequence of the 193-long *Hae*III fragment of Xlo 5. Missing residues denoted by X are methylated Cs situated in *Eco* RII restriction methylation sites¹⁶.

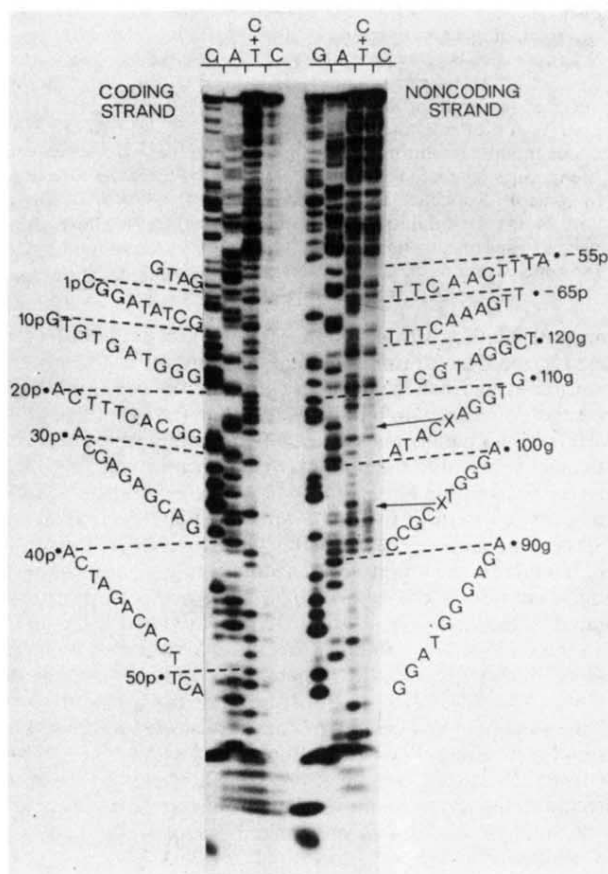
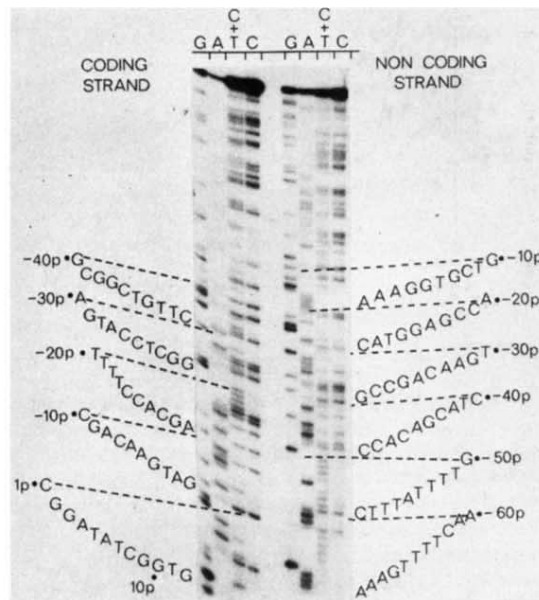
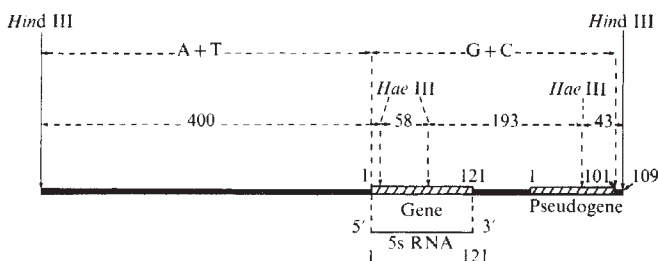


Fig. 1 Location of gene and pseudogene and the *Hae*III and *Hind*III sites in the 5S DNA monomer.



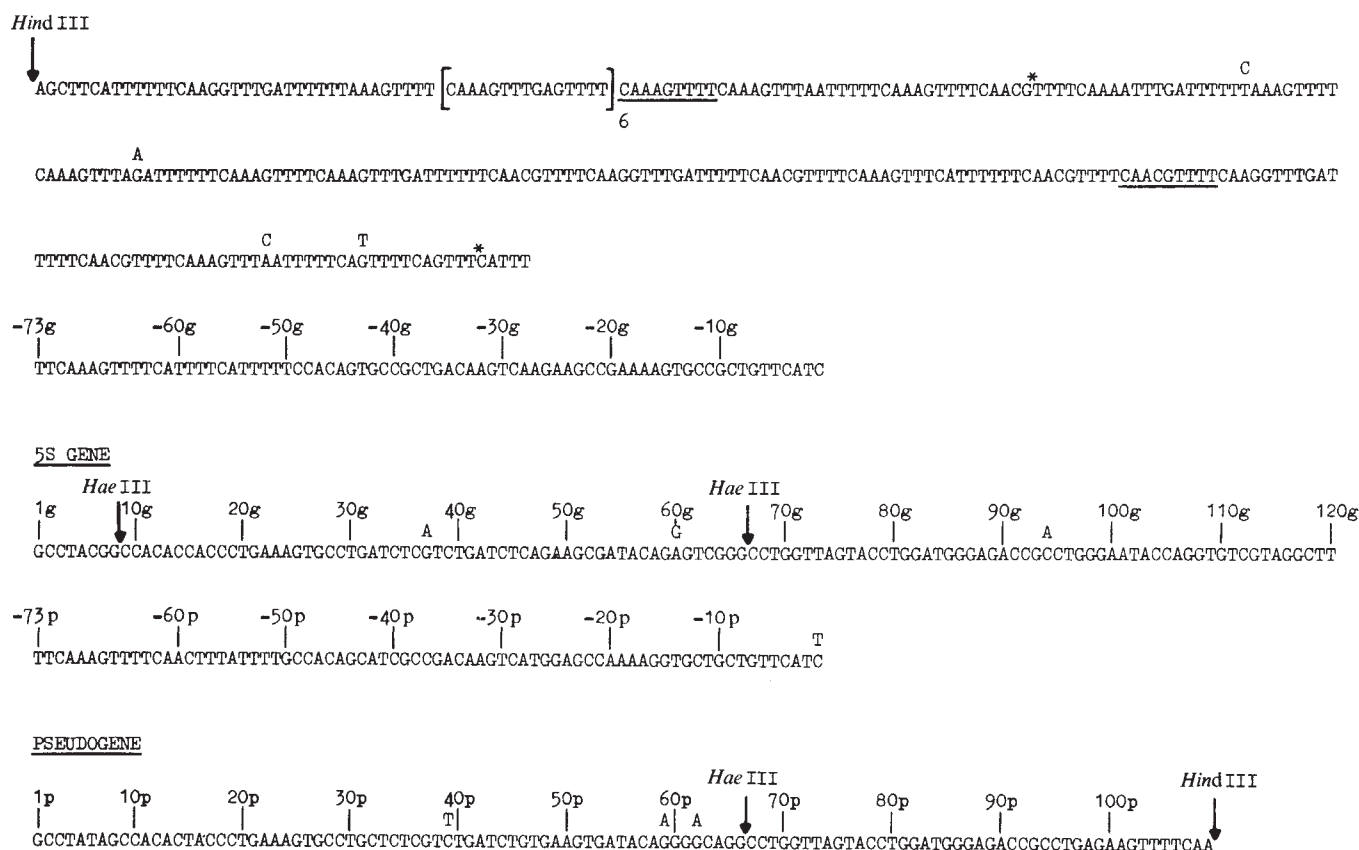


Fig. 3 Comparison of the sequences of clone Xlo 5 and clone Xlo 31. The complete sequence of Xlo 5 is shown with the differences present in Xlo 31 indicated above. The cloned 5S DNA was purified as described previously¹⁶. The sequence shown was obtained by digesting the 5S DNA with the restriction enzyme *Hae*III, producing four fragments, which were then radioactively labelled at their 5' ends using polynucleotide kinase, then strand-separated and sequenced by the Maxam and Gilbert technique¹⁸, all as detailed previously¹⁶. The only variations were that the sequencing gels were the recently developed 'thin' variety¹⁹ and that the 58-long *Hae*III fragment was strand-separated by electrophoresis on a 20% 7 M urea polyacrylamide gel. The sequences of the three smaller *Hae*III fragments were completely determined, as was the sequence of the large A + T rich *Hae*III fragment of Xlo 31. However, there was a region in the middle of this large fragment in Xlo 5 where, because of the distance from either end, sequencing gels could not be read unambiguously. The gels could, however, be read in conjunction with the composite sequence deduced for this region in whole frog¹⁵, and the actual sequence of this fragment in Xlo 31. The sequence deduced, bounded by asterisks, must be an extremely close approximation to the actual sequence present in Xlo 5. The sequence shown here is that of the non-coding strand, hence if T residues were replaced by U residues the gene sequence would yield the sequence of 5S RNA.

in other clones¹⁵ and is generally associated with variations in the number of copies of the oligonucleotide shown in square brackets. There were only two copies in Xlo 31 compared to six copies in Xlo 5. Also there was a T → C shift in the penultimate copy of this same oligonucleotide in clone Xlo 5 as previously noted for other clones¹⁵ giving the variant sequence C-A-A-A-G-T-T-C-G-A-G-T-T-T. In addition there were two block deletions of the underlined oligonucleotides in clone Xlo 31. The second type of microheterogeneity was point mutation: 12 changes were observed, five occurring in the A + T rich spacer and the remaining seven in the G + C rich region. The distribution of mutations amongst the G + C rich region is of interest. Three mutations are in the coding sequence (37g, 60g and 94g), three are in the pseudogene (39p, 59p and 62p) and one between them (-1p).

Apart from the well known^{10,15} length heterogeneity of the A + T spacer caused by additions and deletions, we now see that point mutations occur fairly evenly throughout the entire repeat unit. The G + C region, which includes gene and pseudogene, is at least as heterogeneous as the A + T region. These studies involved two repeats out of a total of about 24,000 and so must be interpreted cautiously. But the result is supported by previous work on oocyte 5S RNA¹²⁻¹⁴. Microheterogeneity occurred at positions 30, 47, 53, 55, 56, 79 and 91 and a mixture of at least four and perhaps eight different sequences was present¹³. The three positions of heterogeneity found here in the gene, namely 37g, 60g and 94g, are different and were presumably below the detection level in the previous RNA sequencing

studies. Taken together these results suggest that there is a distinct and more extensive heterogeneity in the 5S RNA genes than was previously assumed. In fact we could speculate that every single 5S RNA molecule might differ by at least one base. This disagrees with the concept that gene sequences are essentially identical¹¹.

This heterogeneity, though distinct, is far smaller than would be the case if there were no correction mechanism at all in operation. If this were so we would expect an almost complete sequence divergence assuming a mutation rate of 5×10^{-9} mutations per base pair per year¹¹ and a phylogenetic time scale of 350 Myr (ref. 20). Previous studies have shown that the sequence of 49 nucleotides immediately 5' to the gene is conserved in six different cloned repeating units¹⁵. This homogeneity was also preserved in clone Xlo 5. It is no accident that these 49 bases are thought to contain the putative 5S 'promoter' structure^{15,16}. This indicates that there are two different degrees of sequence conservation. A functionally important structure like the presumed promoter has its sequence completely conserved. The remainder of the spacer and also, surprisingly, the 5S gene sequence, seem to undergo a considerably lower level of sequence conservation.

The concept of unequal crossing over is compatible with the observed sequence microheterogeneity. As noted previously¹⁵ the presence of the 15-long oligonucleotide within a larger repeat structure correlates well with the prediction of the development of short and long range periodicities within the A + T spacer region¹¹. We must now assume that natural

selection will eliminate mutations in the promoter regions, whereas a level of heterogeneity in the spacer and gene regions can be tolerated. The extent to which the correction mechanism works on the gene sequences need only be enough to keep the heterogeneity of the 5S RNA in ribosomes within a limit which the animal can tolerate. As the precise function of 5S RNA is unknown, this limit is unclear, but is perhaps not more than a few mutations per the 'averaged' 5S RNA sequence'.

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Splicing of SV40 late mRNA is a post-transcriptional process

THE synthesis and metabolism of SV40 mRNA is being used as a model system to study RNA transcription and processing in animal cells. We and others have recently reported the finding of a novel mechanism of viral RNA maturation in SV40-infected cells¹⁻⁷ in which the leader sequences (those found at the 5' ends of the main bodies of SV40 late mRNAs) and the adjacent coding sequences are transcribed from non-contiguous segments of the viral DNA. Similar observations have been reported for adenovirus mRNAs^{8,9}. This process is known as splicing and may proceed by several possible routes. These include: intermolecular ligation of RNA; deletion in the DNA template of the intervening sequences; looping out of intervening DNA sequences so that the RNA polymerase could skip over short distances; and deletion of the appropriate intervening RNA sequences at the post-transcriptional level. We present here an analysis of R-loop structures¹⁰ formed between nuclear and cytoplasmic poly(A)⁺ RNAs and linear SV40 DNA, which provides support for the last of these possibilities⁴⁰. A similar analysis has been carried out for β -globin mRNA¹⁷.

It has been shown that nuclear poly(A)⁺ viral RNA accumulates in the nucleus of the infected cell (refs 9, 10 and Y.A., M.H., O.L. and Y. Reuveni, submitted for publication). Figure 1 shows that the majority of the poly(A)⁺ viral RNAs present in the nuclei of infected cells sediments in sucrose gradient as 19S components: only a minor fraction sediments faster than 19S and no 16S poly(A)⁺ viral RNA is observed.

R-loops were obtained by annealing nuclear or cytoplasmic 19S poly(A)⁺ RNA with double-stranded linear SV40 DNA obtained by cleavage of form I DNA with *Bgl*I or *Taq*I restriction endonucleases. *Bgl*I and *Taq*I cleave form I DNA at 0.67 and 0.58 map units respectively¹. Figures 2 and 3 show the appearance of representative R-loop structures with their schematic tracings. There are two striking differences in the appearance of the R-loops: (1) the 5' ends of the R-loops pertaining to the nuclear RNAs are closer to the restriction

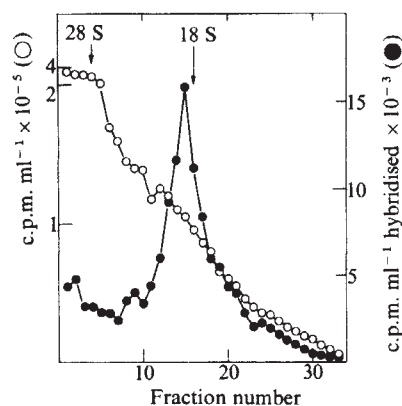
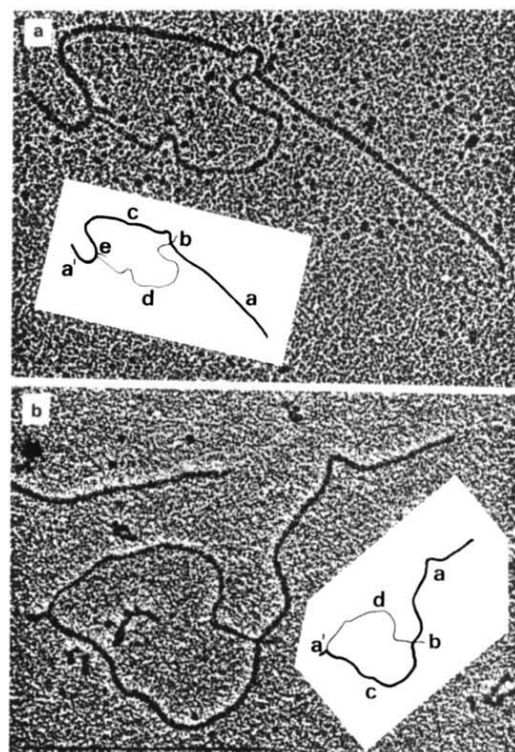


Fig. 1 Sedimentation analysis of nuclear poly(A)⁺ viral RNA. Forty-eight hours after infection of BSC-1 cells with plaque-purified SV40 stock (strain 777), cells were labelled with ³H-uridine for 5 h and labelled nuclear RNA extracted¹³. The ³H-RNA was dissolved in 1 ml of 0.5 M NaCl, 0.01 M Tris-HCl pH 7.4 and fractionated on an oligo-dT-cellulose column (0.5 × 3 cm) as described before¹⁴. Poly(A)⁺ RNA was eluted from the column with 0.01 M Tris-HCl pH 7.4 and collected by ethanol precipitation and centrifugation. Sedimentation analysis of the labelled RNA was performed in 15-30% (w/w) sucrose gradient in SDS buffer¹⁵, centrifuged for 16 h at 25,000 r.p.m. at 20 °C in a Spinco SW27 rotor. At the end of the run, fractions were collected and the radioactivity counted in Triton-based scintillation fluid. Twenty-five-microlitre aliquots from each fraction were hybridised to SV40 DNA immobilised on nitrocellulose filters in 4 × SSC at 68 °C for 20 h, as previously described¹⁰. Fractions 10-20 were collected and used for the R-loop analysis.

Fig. 2 Electron micrographs and their tracings of R-loops composed of linear SV40 DNA (form I cleaved at position 0.67 map units with *Bgl*I restriction endonuclease)¹ and cytoplasmic 19S poly(A)⁺ RNA in *a* or nuclear poly(A)⁺ RNA in *b*. Preparation of the R-loops for electron microscopy was as detailed in ref. 3. In the tracing the letters represent: *a*, double-stranded DNA; *b*, free tail of poly(A); *c*, a heteroduplex DNA-RNA which includes the body of the RNAs; *d*, displaced single-stranded DNA; *e*, free tail of a 'leader'³; *a'*, double-stranded DNA. See text for differences between R-loops pertaining to nuclear and cytoplasmic 19S poly(A)⁺ RNAs. Scale bars = 0.5 μ m.



enzyme cleavage sites than are the 5' ends of those pertaining to the cytoplasmic RNAs; (2) free leaders are often seen at the 5' ends of the R-loops pertaining to the cytoplasmic 19S RNA (in about 70% of the molecules), whereas they are observed much less frequently in the R-loops pertaining to the nuclear 19S viral RNA (in about 10% of the molecules).

Figure 4 is a histogram summarising the map positions of the 5' ends of nuclear and cytoplasmic 19S poly(A)⁺ RNAs. The position of the 5' termini of the cytoplasmic RNA species (dark histogram) is represented by a reasonably sharp histogram with a mean at coordinate 0.77 ± 0.01 . The histogram for the location of the 5' termini of the nuclear viral RNAs (striped histogram) is skewed towards an upstream position and is more heterogeneous. The apparent sharpness of the histogram in nuclear RNA/SV40 DNA (*Bgl*I) (Fig. 4a) results presumably from the fact that the 5' ends of the R-loops are close to the *Bgl*I cleavage site and are therefore less stable. Indeed, 'opened' forked R-loops were observed in this preparation. The small peak at coordinate 0.95 represents a minor contamination with cytoplasmic 16S RNA which is the predominant viral RNA accumulating in the infected cells. The heterogeneity of the location of the 5' ends of nuclear viral RNAs is consistent with a model previously suggested for transcription of prokaryotic DNA¹⁶. Accordingly, several RNA polymerases bind to a short stretch of DNA from which they are released in succession to begin transcription.

The ability of the precursor nuclear poly(A)⁺ viral RNA to anneal to the entire length of the late portion of SV40 DNA (~0.67–0.17 map units) including the corresponding interruptions between the leaders and the bodies of the cytoplasmic mRNAs indicates that splicing is a post-transcriptional process. Splicing of the precursor poly(A)⁺ viral RNA may occur in the nucleus before the RNA molecule is transported to the cytoplasm. It is possible, however, that splicing occurs during the transport from the nucleus to the cytoplasm or immediately after the molecule has reached the cytoplasm. The excision–ligation

Fig. 3 Electron micrographs and their tracings of R-loops composed of linear SV40 DNA (form I cleaved at position 0.58 map units with *Taq*I restriction endonuclease)¹ and cytoplasmic 19S poly(A)⁺ RNA in *a* or nuclear poly(A)⁺ RNA in *b*. See Fig. 2 for explanations.

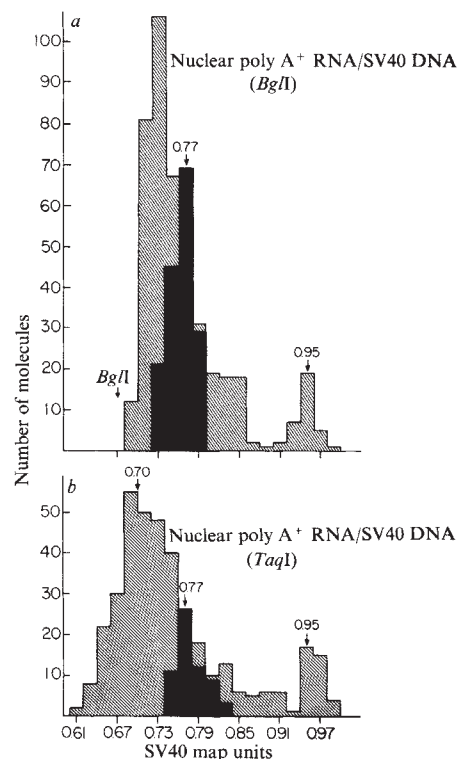
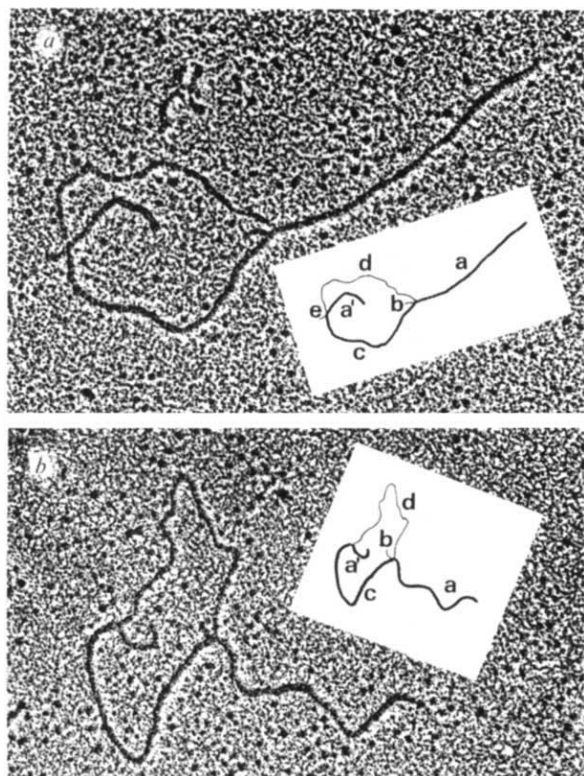


Fig. 4 Histogram of the locations, on the physical map of SV40, of the 5' ends of the bodies of the 19S poly(A)⁺ nuclear and cytoplasmic viral RNAs. The locations of the 5' ends of the bodies of the viral RNAs were determined from measurements of molecules as in Figs 2 and 3. The distances between the 5' end of the R-loop and the restriction enzyme cleavage site (*a'* in the tracing) were measured and expressed for each molecule as the fractional length of the viral DNA. The locations on the physical map of SV40 DNA were determined by adding the value obtained to 0.67 in *a* and to 0.58 in *b*. The dark histogram is cytoplasmic RNA and the striped histogram is nuclear RNA.

reaction in the precursor RNA molecule is carried out, presumably, by an enzyme(s) which recognises short base-paired sequences in the precursor RNA molecule. This reaction would represent a novel mechanism in the regulation of gene expression in animal cells.

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3'-Terminal labelling of RNA with T4 RNA ligase

T4 RNA LIGASE catalyses the formation of an internucleotide phosphodiester bond between an oligonucleotide donor molecule with a 5'-terminal phosphate and an oligonucleotide acceptor molecule with a 3'-terminal hydroxyl¹⁻³. Although the minimal acceptor must be a trinucleoside diphosphate, dinucleoside pyrophosphates and mononucleoside 3',5'-bisphosphates (pNps) are effective donors in the intermolecular reaction⁴⁻⁶. We demonstrate here that various high molecular weight RNA molecules are acceptors in the RNA ligase reaction even when present in very low concentrations in the reaction mixture. One immediate consequence of this observation is that a convenient method for labelling the 3' end of RNA molecules *in vitro* becomes available. By using a [5'-³²P]pNp as a donor and RNA as an acceptor, the product of the reaction is an RNA molecule one nucleotide longer, with a 3'-terminal phosphate and a ³²P-phosphate in the last internucleotide linkage. This reaction is therefore analogous to the *in vitro* labelling of the 5' termini of RNA chains with polynucleotide kinase and [γ -³²P]ATP and can be used in situations where 5' labelling is not possible. In addition, the ability to add various donors to an RNA molecule should allow the function of the 3' terminus of the molecule to be investigated.

Twelve different purified preparations of one or more RNA species were tested for their ability to act as acceptors in the RNA ligase reaction (Table 1). These RNAs varied in chain length from ~870 nucleotides (smallest of the 10 segments of the reovirus genome⁷) to ~15,000 nucleotides (Sendai virus RNA⁸). [5'-³²P]pAp or pCp, the donors in these reactions, were prepared from [γ -³²P]ATP and the corresponding 3' monophosphate using T4 polynucleotide kinase. In extensive studies with oligomers⁵ and tRNA⁹, pCp is found to be the more reactive donor and is, therefore, preferable for 3'-end labelling.

Table 1 3' End labelling of RNA

RNA (source)	Concentration of acceptor in reaction (nM)	Yield (%)
<i>E. coli</i> 16S rRNA (C. Cantor)	120	42
<i>E. coli</i> 23S rRNA (D. Shapiro)	40	18
<i>E. coli</i> 23S + 16S rRNA (S. Rose)	200	44
Rabbit reticulocyte 18S rRNA (D. Shapiro)	110	24
Human 28S rRNA (B. Coté)	40	39
Satellite tobacco necrosis virus (STNV) RNA (J. Clark, Jr)	180	93
Brome mosaic virus RNA 1-4 (P. Kaesberg)	85	77
Brome mosaic virus RNA 4 (P. Kaesberg)	220	54
Q β virus RNA (P. Cole)	60	25
Sendai virus RNA (D. Kolakofsky)	12	107
Reovirus RNA (A. Shatkin)	130	8
Ovalbumin mRNA (D. Shapiro)	100	114

Reactions were carried out in a total volume of 30 μ l in the following conditions: 1 μ g RNA (12-220 nM), [5'-³²P]pAp (1 μ M, 850-1,520 Ci mmol⁻¹), ATP (5 μ M), HEPES (50 mM, pH 8.3), MgCl₂ (10 mM), dithiothreitol (3.3 mM), dimethyl sulphoxide (10%, v/v), glycerol (15%, v/v) and RNA ligase (400 units ml⁻¹, 200 μ g ml⁻¹), and incubated at 4 °C for 24 h. (The RNAs were provided by those named in parentheses.) Yields were determined from the amount of radioactivity precipitable on a nitrocellulose filter in 5% trichloroacetic acid (TCA), 100% reaction equalling the number of mols of RNA added. However, as several samples were contaminated with smaller RNAs their values generally represent an overestimate of the yield. For example, high levels of incorporation were not expected for STNV RNA, as about 50% of the 3' ends are blocked with a phosphate¹⁸. Analysis on a 20% acrylamide gel indicated that the majority of TCA-precipitable label was incorporated into oligomers less than 80 nucleotides in length.

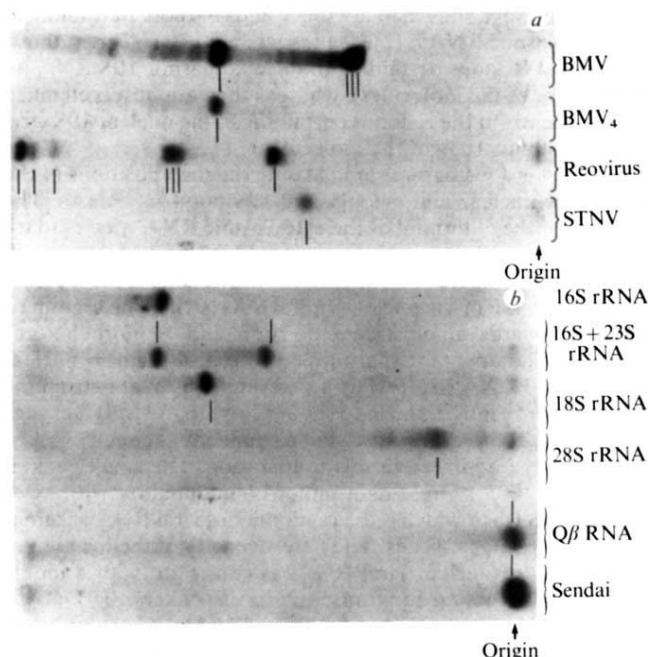


Fig. 1 Analysis of RNA ligase reactions by vertical, denaturing polyacrylamide gel electrophoresis. Aliquots from reactions in 9 M urea-glycerol (1:1, v:v) were loaded on to acrylamide slab gels (19:1, acrylamide:bisacrylamide; 3 mm $\times$ 22 cm) containing 7 M urea, 90 mM Tris-borate (pH 8.3) and 10 mM EDTA¹⁹ and electrophoresed at 250 V at 25 °C with the borate-EDTA as running buffer. In adjacent wells, 1-3 μ g of the unreacted RNAs were run. Radioactive bands were visualised by autoradiography. The lines indicate the position of the unreacted RNAs visualised with 'Stains-All'. a, BMV₍₁₋₄₎, BMV₄, reovirus and STNV were electrophoresed for 16 h on a 4% acrylamide gel. b, 16S rRNA, 23S plus 16S rRNA, 18S rRNA, 28S rRNA, Q β and Sendai were electrophoresed for 20 h on a 2.5% acrylamide gel.

Each reaction contained 1 μ g of RNA, a substantial excess of donor and ATP, and a high concentration of RNA ligase (5 μ M). A low temperature (4 °C) and long incubation time (> 16 h) were chosen to minimise the rapid loss of RNA ligase activity which occurs at higher temperatures^{5,10}. Finally, we have found that the extent of reaction is increased two- to threefold⁵ by including 10% dimethyl sulphoxide in the reaction mixture.

Analysis of the yields by acid precipitation of the RNA and Millipore filtration indicated that although all 12 RNA preparations were acceptors, the efficiency of the reaction varied considerably. Differences in the concentration of 3' termini cannot be the only cause for this, as no correlation between yield and concentration was observed. The sequence and the secondary structure at the 3' end of the RNA are also thought to affect the reaction yield. Comparison of oligonucleotide acceptors indicates that the composition of the terminal three nucleotides has a strong influence on yield⁵. The known specificity of RNA ligase for single-stranded RNA¹¹ can account for the low yield encountered for the 'blunt', double-stranded end of reovirus RNA and suggests that a 3'-terminal secondary structure could explain the comparatively low yields with other RNAs.

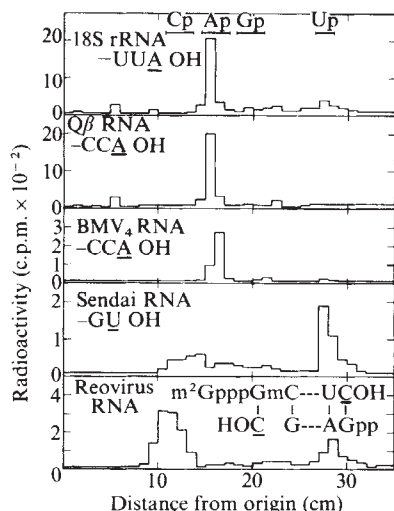
In Fig. 1, the products of the reactions in Table 1 are analysed by acrylamide gel electrophoresis in 7 M urea, where the secondary structure of RNA is minimised. Autoradiography of the gels reveals that the major ³²P-labelled RNA bands migrate closely with the major staining bands. This observation supports the conclusion that the [5'-³²P]-labelled nucleotide bisphosphate has been added on to the 3' end of the RNA. When a mixture of RNA molecules was used in a reaction (*Escherichia coli* rRNA, brome mosaic virus (BMV) and reovirus) all species were labelled to a similar extent. In addition, contaminating RNAs present in the reaction mixture are also labelled. In several of these cases, the smaller contaminants have run off the end of the gel and can only be detected with higher percentage

gels. However, we can exclude fragmentation of the product during the ligation reaction, as smaller RNAs are not observed in all cases.

In several of the reactions in Table 1, the ^{32}P -labelled RNA products have been purified from the unreacted bisphosphate and any low molecular weight impurities by Sephadex G-100 column chromatography. Complete digestion of these RNAs with a mixture of ribonucleases A, T_1 and T_2 and analysis of the products by high-voltage paper electrophoresis give a single ^{32}P -labelled nucleoside monophosphate corresponding to the known 3'-terminal nucleotide of the RNA molecule (Fig. 2). In the case of double-stranded reovirus RNA, analysis of a T_1 digest of the labelled RNA indicates that the 3' ends of both the plus and the minus strands were being labelled to a similar extent (A. Shatkin, personal communication). Finally, it is important to note that no reaction occurs at the 3' hydroxyl of m⁷G of the 'cap' structures on reovirus RNA, BMV RNA or ovalbumin mRNA. It is unlikely that cap structures will be substrates for RNA ligase, as a trinucleoside diphosphate is required as a minimal acceptor^{2,5} and isolated Ado5'ppp5'Guo was found to be inactive as a donor⁴.

The use of T4 RNA ligase to label the 3' end of RNA *in vitro* offers several advantages over other methods of *in vitro* labelling of RNA. First, the 3' ends of RNA molecules are usually free 3' hydroxyl groups and therefore can be used directly as acceptors for RNA ligase. In contrast, labelling the 5' ends of RNA with T4 polynucleotide kinase is often complicated by the presence of one or more 5'-terminal phosphates or the 'cap' structure, which must be removed before the labelling reaction can be carried out. Second, as endonucleolytic breakdown products of RNAs will often have a 3'-terminal phosphate and a 5'-terminal hydroxyl, they will be labelled by polynucleotide kinase but not by RNA ligase. Thus, RNA ligase reactions will have fewer radiolabelled contaminants. Third, in contrast to other *in vitro* procedures that are limited to a single isotope^{12,13}, nucleoside 3',5'-bisphosphates may be prepared as 5'- ^{32}P or with ^3H or ^{14}C in the nucleoside. (^{125}I)5-iodocytidine 3',5'-bisphosphate is also a donor in the RNA ligase reaction⁹. Finally, as preparations of RNA ligase can be made free of nucleases and the reaction conditions are extremely mild, the labelled RNA can be recovered intact in high yields.

Fig. 2 Nearest-neighbour analysis of 3'-end labelled RNA. The RNAs from ligation reactions were purified by gel filtration on Sephadex G-100, ethanol-precipitated and degraded completely with a mixture of RNases A, T_1 and T_2 according to Barrel²⁰. The 3' mononucleotides were resolved by paper electrophoresis on Whatman no. 1 in 0.025 M Na citrate, pH 4.0, at 4,500 V for 2 h (ref. 21). The sequences of the 3' termini of 18S rRNA, Q β , BMV₄, Sendai and reovirus have been reported elsewhere (refs 22, 23, 17, 24, 25, respectively).



RNA with a radiolabel at the 3' end may be used to determine the sequence of nucleotides near the label by one of several recently developed methods¹⁴⁻¹⁶. For example, 15 nucleotides of the 3'-terminal sequence of BMV-4 RNA were confirmed¹⁷ with material prepared in this work by using the two-dimensional 'wandering-spot' sequencing procedure (R. Dasgupta and P. Kaesberg, personal communication). Thus, 3'-terminal labelling with RNA ligase complements 5'-terminal labelling with polynucleotide kinase, and the two methods may be used to study the sequence at both ends of a molecule and, if the molecule is short enough, confirm the results of the other method.

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Cloning of replication origins from the *E. coli* K12 chromosome

MINIPLASMIDS, containing only an origin of replication and plasmid-coded functions necessary for plasmid replication and maintenance have been isolated from larger plasmids¹. The technique used was to cleave the parent plasmid with the restriction endonuclease *EcoRI* and reanneal and ligate the fragments so generated in the presence of another *EcoRI*-generated fragment of DNA coding for a β -lactamase. This second fragment was not capable of self-replication but hybrids between it and a plasmid fragment which was able to replicate could be detected because calls of *Escherichia coli* transformed with such hybrids would be resistant to ampicillin. We have used the same technique to isolate minichromosomes from the total chromosomal DNA of *E. coli* K12 and have found that two different chromosomal *EcoRI* fragments can function as autonomously replicating plasmids. One type of plasmid is indistinguishable from that isolated independently by Yasuda and Hirota² and contains the origin of replication normally active in chromosome replication termed *oriC* (refs 3-6). The other type, which we call *oriJ* plasmids to distinguish them from

oriC plasmids, are described below. Both types can be isolated from a single strain of *E. coli* and they contain chromosomal DNA which is not homologous in DNA-DNA hybridisation studies. Consequently we conclude that the *E. coli* K12 chromosome contains at least two segments of DNA capable of self-replication.

The fragment of DNA coding for β -lactamase (the Ap fragment) used in our study was obtained from an amplifiable ColE1-Ap plasmid (pLG1) which was constructed by *in vivo* ligation of the Ap fragment (4.5 megadaltons) of the plasmid pSC122 (ref. 1) with Col E1 DNA which had been cleaved at its single *EcoRI* site. ColE1-Ap amplified by incubation of its host in chloramphenicol⁷ was purified⁸ and cleaved with *EcoRI* and the Ap fragment separated in an equilibrium density gradient of CsCl, making use of a density difference between it ($\rho = 1.693$) and the linear form of ColE1 ($\rho = 1.710$) (ref. 7).

In one experiment, an Ap fragment preparation (1 μ g) was annealed and ligated⁹ with an *EcoRI* digest of *E. coli* K12 DNA (3 μ g) isolated from strain C600 (ref. 10) by Marmur's method¹¹. After dialysis, the ligation mixture was used to transform¹³ the F⁻ *recA* strain AB2463 (ref. 12), and ampicillin resistant transformants were selected on appropriately supplemented M9 synthetic medium¹⁴ containing ampicillin (40 μ g ml⁻¹). Before plating the transformed cells were incubated in LB broth¹⁵ for 90 min at 37 °C to permit expression of the β -lactamase gene. Of 1,600 ampicillin-resistant colonies which emerged, 1,000 were tested and 5 found to be sensitive to colicin E1. Three of these gave ampicillin-sensitive segregants, and were found to contain plasmid DNA when total lysates¹⁶ were analysed in CsCl-ethidium bromide equilibrium density

gradients¹⁷. DNA isolated from C600, AB2463, and a pooled extract from 10 ampicillin-sensitive segregants of one ampicillin resistant clone, contained no detectable plasmid DNA.

Each of the three plasmids from the ampicillin-resistant, colicin-sensitive clones gave two *EcoRI* cleavage fragments, one of 4.5 megadaltons corresponding to the Ap fragment and another of 8.3 megadaltons. They seemed to be identical on the basis of subsequent analysis with *HindIII*. One of them, called *oriJ*-Ap (pLG2), was subjected to further analysis. Interestingly, none of these first three plasmids had an *EcoRI* fragment of 5.9 megadaltons as reported for the *oriC* plasmids. The 8.3 megadaltons fragment is not a derivative of ColE1 (4.2 megadaltons), the only other replicon known to be involved in our protocol, and is not derived from contaminating DNA. Both alternatives were ruled out by DNA-DNA hybridisation using a modification¹⁸ of Southern's¹⁹ method. *oriJ*-Ap DNA was labelled to high specific activity with ³²P by nick-translation¹⁸. This DNA was then used as a probe to test for hybridisation against *EcoRI*-digested ColE1 DNA, Ap DNA, *EcoRI*-digested *oriJ*-Ap DNA, an *EcoRI* digest of *E. coli* DNA from strain C600 and an *EcoRI*-digest of bacteriophage λ CI857 DNA. No hybridisation occurred between ColE1 and *oriJ*-Ap (Fig. 1a, b) indicating that they contain no extensive sequence homology and thus that the latter is not derived from the former. As a control it can be seen that both *EcoRI* fragments of *oriJ*-Ap (Fig. 1e, f) and the Ap fragment (Fig. 1c, d) hybridised with *oriJ*-Ap DNA. Figure 1g, h shows that *E. coli* DNA contains an *EcoRI* fragment 8.3 megadaltons in size which hybridises with *oriJ*-Ap. On the other hand (data not shown) the Ap fragment itself shows no hybridisation with *E. coli* DNA, hence the hybridisation seen in Fig. 1h must be between *E. coli* DNA and the 8.3 megadalton fragment of *oriJ*.

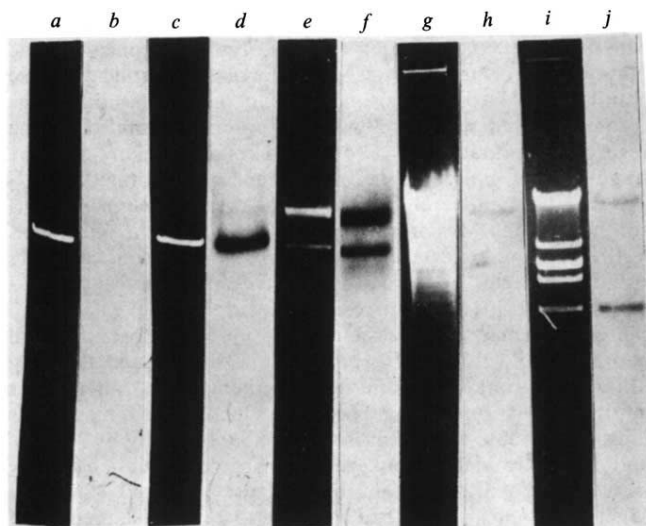
The two *EcoRI* fragments of bacteriophage λ CI857 DNA which contain the cohesive ends²⁰ gave a faint reaction with the *oriJ*-Ap probe but no hybridisation was detected with the 4.7-megadalton fragment which contains the origin of phage replication²⁰ (Fig. 1, track j). Note that in Fig. 1 the exposure time for track j was 8 times longer than for other tracks. The significance of the faint reaction is not clear but the absence of homology with the 4.7-megadalton fragment suggests that *oriJ* is not derived from λ DNA which might be present in the C600 chromosome.

A further study of the structure of *oriJ*-Ap was made by a combination of restriction analysis with *EcoRI* and *HindIII* enzymes and hybridisation with a ³²P-labelled Ap DNA probe. The results obtained are shown in Fig. 2 and confirm that the 4.5-megadalton *EcoRI* fragment of *oriJ*-Ap is the Ap fragment used in the cloning experiment, and are consistent only with the restriction map shown in Fig. 2B or with one in which the order of the 2.4- and 1.4-megadalton segments is reversed. Restriction analysis with *Bam*H1 enzyme (data not shown) confirm the order given in Fig. 2B.

To compare the restriction map of *oriJ* with that of the homologous 8.3-megadalton fragment of C600 chromosomal DNA, ³²P-labelled *oriJ*-Ap was hybridised against C600 DNA digested with *EcoRI*, *HindIII* or both endonucleases (Fig. 2C). An analysis of the molecular weights of the major hybridisation bands shows that the 8.3-megadalton fragment present in the C600 chromosome generates a family of *HindIII* fragments indistinguishable from the fragments generated by the 8.3-megadalton component of *oriJ*-Ap. Thus it seems that a single segment of chromosomal DNA has been incorporated into *oriJ*-Ap probably without major structural rearrangements. The data in Fig. 2C also provide information about the location of the two *HindIII* cleavage sites adjacent to the 8.3-megadalton segment and these are indicated in Fig. 2D.

It is conceivable that different K12 strains have different restriction target maps in the neighbourhood of the chromosome origin and that *oriJ* and *oriC* both contain the same origin of replication. Strain KL228 (ref. 21) is an Hfr from which F-prime derivatives have been isolated that carry a 5.9-megadalton *EcoRI* fragment inside a restriction map which is

Fig. 1 Homology studies with *oriJ*-Ap DNA. *oriJ*-Ap (0.5 μ g) was labelled with ³²P (1–5 $\times 10^6$ cpm μ g⁻¹) by nick-translation¹⁸. This probe was hybridised^{18,19} to DNA (0.1–0.15 μ g) that had been previously digested to completion with *EcoRI*, fractionated by electrophoresis (90 min at 150 V) in 0.8% agarose containing Tris-borate-EDTA buffer²⁹, denatured in the gel by treatment with alkali and finally blotted onto nitrocellulose filters. Hybridisation was carried out in 3 $\times$ SSC at 65 °C for 2 d. The filters were given a post-hybridisation wash in 3 $\times$ SSC followed by 0.1 $\times$ SSC at 65 °C. Hybridisation bands were detected by autoradiography using a Kodak X-Omat film that was exposed to the filters at –80 °C for 18 h (tracks b, d, f, h) or 6 d (track j). The ethidium-bromide pattern (left) and the corresponding DNA material that hybridises with the probe (right) are shown in pairs: tracks (a) and (b) ColE1 DNA $\times$ *EcoRI*, (c) and (d) Ap fragment, (e) and (f) *oriJ*-Ap $\times$ *EcoRI* (control), (g) and (h) C600 DNA $\times$ *EcoRI* and (i) and (j) λ CI857 DNA $\times$ *EcoRI*. (Molecular weights of λ fragments: 13.7, 4.7, 3.7, 3.5, 3.0 and 2.0 megadaltons respectively; 3.7 and 3.5 megadalton fragments are not resolved in the gel).



indistinguishable from that of the 5.9-megadalton fragment of *oriC* (ref. 4). When *oriJ*-Ap was used as a probe to test for hybridisation with an *EcoRI* digest of DNA from KL228, no hybridisation was found in the 5.9-megadalton region (Fig. 2C, track d). On the contrary, the hybridisation pattern against the *EcoRI* digest and a *HindIII* digest (Fig. 2A, d and e) of this DNA indicates that it contains the *oriJ* segment. Thus KL228 probably contains both the *oriC* and *oriJ* segments.

If the two plasmids are derived from distinct regions of the K12 chromosome then both should be recoverable in a single DNA preparation. The fact that none of the three ampicillin resistant clones isolated by us contained *oriC* plasmids whereas all three such clones isolated in a similar way by Yasuda and Hirota did so, suggested that the choice of recipient strain might

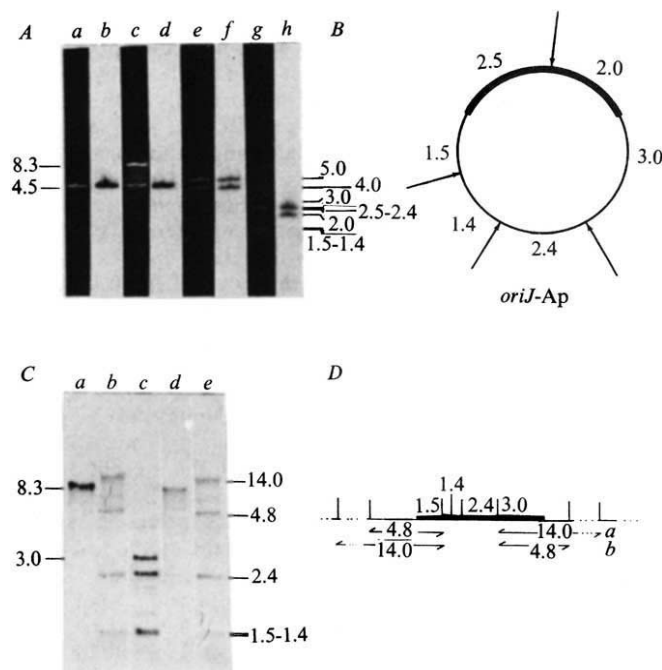
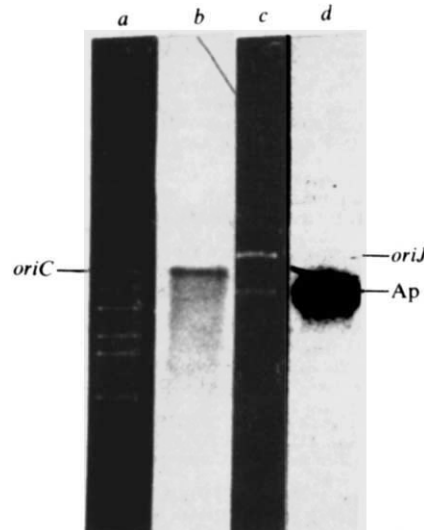


Fig. 2 Physical analysis of *oriJ*-Ap and of the homologous *E. coli* K12 chromosomal region. **A**, Combined restriction and hybridisation analysis of *oriJ*-Ap. *oriJ*-Ap (0.3 μ g) was digested with *EcoRI*, *HindIII* or both enzymes, and the products fractionated by electrophoresis in agarose gel and hybridised with Ap DNA (0.5 μ g) labelled with 32 P as indicated in Fig. 1. The Ap fragment (0.1 μ g) was also included in the gel as a control for subsequent hybridisation with this probe. The ethidium bromide pattern (left) and the corresponding DNA material that hybridises with the probe (right) are shown in pairs. Tracks (a) and (b), Ap fragment (control); (c) and (d), *oriJ*-Ap $\times$ *EcoRI*; (e) and (f), *oriJ*-Ap $\times$ *HindIII*; (g) and (h), *oriJ*-Ap $\times$ *EcoRI* $\times$ *HindIII*. Molecular weights of the fragments are given in megadaltons and were calculated using λ cI857 DNA $\times$ *EcoRI* fragments as standard. **B**, Physical map of the *oriJ*-Ap plasmid based on data in (A). Broad curve, Ap fragment; thin curve, chromosomal material; arrows, *HindIII* restriction target. The two *EcoRI* targets are at the two junctions of the Ap fragment and the chromosomal DNA. **C**, Hybridisation analysis of *E. coli* DNA with *oriJ*-Ap. *oriJ*-Ap (0.5 μ g) was used as a 32 P-labelled probe to test for hybridisation with DNA from *E. coli* strains C600 and KL228 which had been digested with *EcoRI* and/or *HindIII* enzymes. All operations were basically as described in Fig. 1 except that the electrophoresis was run for 12 h at 20 V. Tracks (a), (b) and (c) show respectively hybridisation found against C600 DNA digested with *EcoRI*, *HindIII*, and both enzymes. Tracks (d) and (e) show hybridisation found in KL228 treated with *EcoRI* and *HindIII* respectively. Molecular weights of the hybridisation bands are given in megadaltons and were calculated as before. **D**, Two alternative *HindIII* maps (a and b) of the homologous region to *oriJ* in the *E. coli* chromosome based on data in (C) assuming that the orders of the fragments in the chromosome and the *oriJ* probe are identical (thick line).

be important. In particular it seemed possible that the use of an Hfr strain as recipient might favour the isolation of *oriC* plasmids because in such strains the predicted interference of such a plasmid with chromosome replication as a consequence of control of copy number²² might be alleviated because chromosome replication could be driven by the integrated F factor^{23,24}. We also noticed that on rich medium there was selection against colonies containing the *oriJ*-Ap plasmid. We therefore prepared a new ligation mixture of *EcoRI* digested C600 DNA (1.5 μ g) and Ap DNA (1.5 μ g), transformed the Hfr LC248 (ref. 2) with it and selected ampicillin-resistant colonies on LB medium containing 20 μ g ml⁻¹ ampicillin as indicated by Yasuda and Hirota². Five colicin E1 sensitive clones which segregated ampicillin sensitive cells were found among 300 ampicillin resistant colonies. Plasmid DNA (pLG6) isolated from one of these colonies chosen at random was analysed in the manner described for *oriJ*-Ap. It was found to be indistinguishable from *oriC* on the basis of its distribution of *HindIII* and *EcoRI* sites and homologous DNA fragments of similar size were found in C600 DNA. In addition, the sizes of the fragments which hybridise with *oriC* are those predicted if the *HindIII* sites adjacent to the 5.9-megadalton *oriC* fragment are at the locations found by Marsh and Worcel³. Thus we conclude that pLG6 is probably identical to *oriC*. This conclusion is confirmed by the data shown in Fig. 3 which also demonstrate that *oriC* and *oriJ* do not cross-hybridise with each other. In this experiment, DNA from the bacteriophage λ asn 132 (ref. 25), a hybrid λ which contains a segment of chromosomal DNA including an origin of replication within a 5.9-megadalton *EcoRI* fragment^{25,26}, was cleaved with *EcoRI* and the *EcoRI* fragments tested for homology with pLG6 by hybridisation. An *EcoRI* digest of *oriJ*-Ap was included in the same experiment. It can be seen that pLG6 hybridises with the 5.9-megadalton fragment of λ asn 132 (Fig. 3a, b), thus confirming that pLG6 is homologous with the *oriC* region as defined by others^{2,3,25,26}. In addition, there is no hybridisation between pLG6 DNA and the 8.3-megadalton fragment of *oriJ* indicating that the two origin plasmids share no significant homology (Fig. 3c, d).

Fig. 3 Hybridisation analysis of λ asn 132 and *oriJ*-Ap with *oriC*-Ap. *oriC*-Ap was labelled with 32 P and used as a probe to test for hybridisation with λ asn 132 DNA (0.3 μ g) digested with *EcoRI* and *oriJ*-Ap DNA (0.2 μ g) digested with *EcoRI*, basically as described in Fig. 1. λ asn 132 was isolated from strain CM848 (*E. coli* K12 F⁺, *thi. asn* (λ asn 132, λ cI857 S7); source K. von Meyenburg) using published methods³⁰. Tracks (b) to (d) show, in pairs, the ethidium bromide pattern (left) and the corresponding hybridisation picture (right): (a) and (b) λ asn 132 DNA $\times$ *EcoRI* (c) and (d) *oriJ*-Ap $\times$ *EcoRI*. The hybridisation pattern found in the *oriC* region was obtained after 3 h exposure of the autoradiograph and the one found in the Ap region after 60 h exposure. Molecular weights of the λ asn 132 *EcoRI* fragments (megadaltons): 8.2, 6.0, 4.7, 3.7 (3.5), 2.5, 2.0, 1.2.



In relation to the location and functions of the segment of chromosomal DNA which gives rise to *oriJ* plasmids, it is pertinent to note that several authors^{27,28} have postulated the existence of a cryptic prophage integrated in the *sbvA-rac* region of the *E. coli* chromosome and that interestingly the strain that we used as a host for *oriJ*-Ap lacks this prophage. It is noteworthy to mention in this respect that we found a drastic reduction in the transformation frequency with *oriJ*-Ap in some other strains of *E. coli* K12. A full structural analysis of the *sbvA-rac* region in C600 and AB2463 strains and a study of the replication of *oriJ*-Ap in these hosts is in progress.

It is not yet clear to what extent the efficiency of isolation of *oriC* plasmids is influenced by the use of an Hfr recipient as opposed to an F⁻. We have observed that the Hfr recipient LC248 and the F⁻ recipient AB2463 are transformed with similar efficiency by *oriC*-Ap with selection on either M9 or LB media but that the *oriC* plasmid caused a much more severe inhibition of growth of the F⁻ strain than of the Hfr particularly on M9 medium. The basis of these differences in growth rate are being analysed in more detail and in particular the hypothesis that chromosome replication is driven by the inserted F plasmid in Hfr *oriC* strains is being tested directly.

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Stereochemistry of phosphoryl group transfer using a chiral [¹⁶O, ¹⁷O, ¹⁸O] stereochemical course of alkaline phosphatase

THE recent synthesis and stereochemical analysis of a phosphate monoester chiral at phosphorus by virtue only of the three stable isotopes of oxygen^{1,2} opens the way to the determination of the stereochemical course of both chemical and enzymatic reactions that involve the transfer of a phosphoryl group. We report here the first determination of the stereochemical consequence at phosphorus of an intermolecular phosphoryl transfer reaction. In studying the reaction catalysed by alkaline phosphatase we had two aims: first, to discover the stereochemical course of this well-studied transfer, and second to devise a general method by which the configuration of any chiral phosphate monoester can be determined.

The alkaline phosphatase from *Escherichia coli* is a nonspecific phosphate monoesterase³, and there is good evidence that the readily-formed phosphoryl enzyme is a reaction intermediate in the mechanistic pathway normally followed by this enzyme³. As with many other enzymes that catalyse hydrolytic reactions, alkaline phosphatase readily catalyses the transfer of phosphoryl groups to acceptors other than water, such as alcohols⁴. As our method for determining the absolute configuration of a chiral [¹⁶O, ¹⁷O, ¹⁸O]phosphoryl group requires its attachment to one enantiomer of propane-1,2-diol, this compound was used as the acceptor in the present experiments.

Phenyl[(R)-¹⁶O, ¹⁷O, ¹⁸O]phosphate was prepared as the crystalline dicyclohexylammonium salt by the method outlined in Fig. 1, which closely parallels that used earlier to prepare [1(R)-¹⁶O, ¹⁷O, ¹⁸O]phospho-(S)-propane-1,2-diol¹. The disodium salt of chiral phenyl phosphate (270 mg) was dissolved in water (4.0 ml) containing KHCO₃ (1.2 mmol), K₂CO₃ (1.2 mmol), Zn(OAc)₂ (30 nmol), and Mg(OAc)₂ (3 μmol). (S)-Propane-1,2-diol (4.0 ml) was added, and the reaction initiated with alkaline phosphatase (10 units *E. coli* enzyme, Sigma). When 49% of the phenyl phosphate had been solvolysed, the reaction was stopped by the addition of Dowex 50 (H⁺ form). The mixture of 1- and 2-phosphopropane-1,2-diols was isolated by ion-exchange chromatography on DEAE-cellulose and on Dowex 1 in 30% yield (based on the phenyl phosphate consumed). This material comprised the 1- and 2-isomers in 9:1 ratio, from which the 1-isomer of phosphopropane-1,2-diol was obtained (containing <3% of the 2-isomer) by crystallisation of the dicyclohexylammonium salt from acetone–water at –20 °C. The isotopic composition of the peripheral phosphate oxygens was: ¹⁶O, 43.7%; ¹⁷O, 15.3%; and ¹⁸O, 41%. These percentages were identical (to within 1%) with the isotope ratios for the peripheral oxygens of the phenyl phosphate starting material.

The absolute configuration of the product 1-[¹⁶O, ¹⁷O, ¹⁸O]phosphopropane-1,2-diol was then determined by the sequence described earlier¹ (this involves ring closure, methylation of an exocyclic oxygen, separation of the resulting diastereoisomeric triesters, methanolytic ring opening, and linked-scan metastable ion mass spectrometry). The configuration at phosphorus in the product was found to be R (92% ± 6%), showing that the phosphoryl group has been transferred with overall retention of configuration at phosphorus.

Of all enzymes that involve the transfer of a phosphoryl group (including phosphatases, phosphokinases and phosphomutases), the alkaline phosphatase from *E. coli* is one of the most thoroughly investigated. The evidence that the isolable phosphoryl enzyme is indeed an intermediate on the normal reaction pathway is good³, and the process is evidently of the 'double-displacement' class⁵. It is therefore gratifying to find

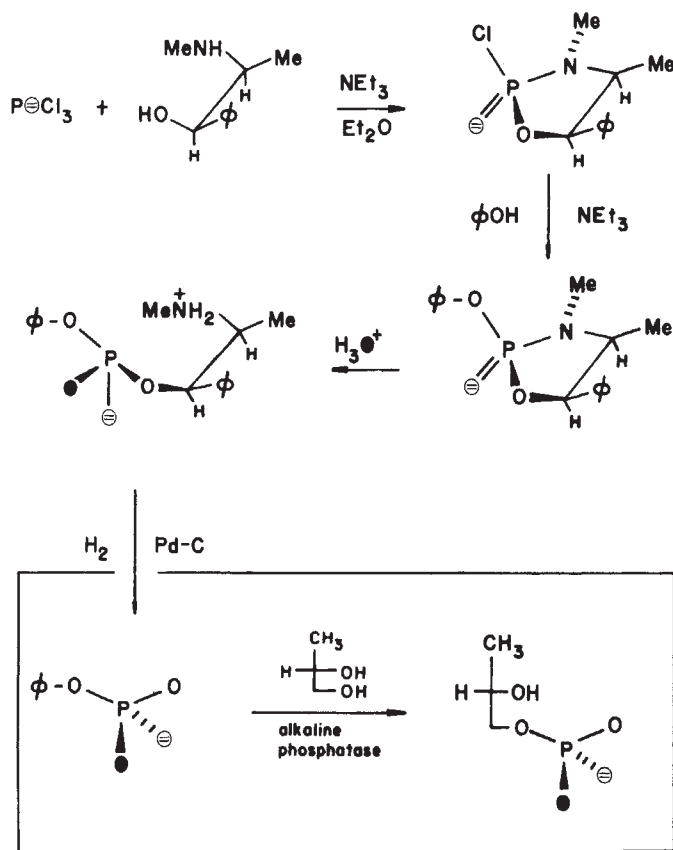


Fig. 1 Synthesis of phenyl[(R)-¹⁶O, ¹⁷O, ¹⁸O]phosphate and enzyme-catalysed transfer of the chiral phosphoryl group to (S)propane-1,2-diol.

that the stereochemical consequence of this reaction is indeed retention. While we cannot yet define the stereochemical course of each of the two single displacement steps (the formation of the phosphoryl-enzyme and its collapse), the probability that the phosphorylation is effectively (even if not precisely) the microscopic reverse of the dephosphorylation, makes overall retention the anticipated result. Further, this work in principle allows the absolute configuration of any chiral [¹⁶O, ¹⁷O, ¹⁸O]phosphate monoester to be determined, facilitating the definition of the stereochemical course of phosphoryl transfer reactions that are mechanistically ambiguous.

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Nanosecond transient Raman spectra of photolysed carboxyhaemoglobin

CONFORMATIONAL CHANGES accompanying the photolysis of carboxyhaemoglobin (CO-Hb) have been the subject of much recent study. It is known from time resolved absorption studies that the process is not simple, comprising various steps on time scales ranging from picoseconds to milliseconds¹⁻⁶. Removal of the CO molecule is complete within picoseconds⁵, but the existence of an unknown transient with a 100 ns lifetime has been demonstrated¹. No conclusion has been possible as to the time required for the reorganisation of the porphyrinato core. In principle, resonance Raman scattering offers a more detailed source of conformational information. From steady state Raman spectra, the normal modes have been identified^{7,8}. A clear empirical correlation has been found⁹ between the position of the resonantly enhanced anomalously polarised line in the region 1,550–1,590 cm⁻¹, and the porphyrinato core size. Here we report the first nanosecond time-resolved spectra of CO-Hb. From our results it is clear that the main reorganisation of the porphyrinato core occurs within 5 ns of photolysis.

Human haemoglobin was prepared according to the standard methods. It was reduced with sodium dithionite and then isolated in 0.01 M phosphate buffer (pH 7) by Sephadex chromatography. This stock solution was stored as CO-Hb under an oxygen-free atmosphere. The samples of CO-Hb diluted from this stock solution were sealed under ~1 atm CO. Solutions of deoxy-Hb were sealed under an N₂ atmosphere after the samples had been rendered free of both O₂ and CO in photolytic conditions. The concentration of the samples, measured using optical absorption in the region 5,300–5,700 Å, was typically 0.1–0.3 mM haem.

The spectra were recorded on an optical multichannel analyser of our own design, which has been previously described¹⁰. The time resolution of this apparatus has been increased by more than four orders of magnitude by the use of the pulsed Nd:YAG laser described below. A spectral region up to 200 cm⁻¹ wide may be observed, thereby eliminating problems associated with shot-to-shot variation of laser power when pulsed lasers are used. When required, it is possible to gate the input slit using a Kerr cell with a switching time of 3 ns. The time resolution is then improved to ~5 ns. In the spectra reported here, the input slit was set at 250 μm, corresponding to a spectral resolution (FWHM) of 6 cm⁻¹. In all cases the spectrometer was calibrated as to absolute position by measurements on the 1,588 cm⁻¹ line of benzene to eliminate backlash effects in the gratings.

Two excitation sources were used. The pulsed spectra were excited by a Nd:YAG laser, frequency-doubled to provide nominal 20 mJ pulses of 5,320 Å radiation with a duration of 10 ns, and a pulse repetition rate of 10 ps⁻¹. In order to obtain cw spectra in similar resonance conditions, a Kr⁺ laser was used, operating at 5,308 Å, at a power of 10–20 mW. The cw beam was sharply focused (~100 μm), while, to avoid damage to the sample container, it was necessary to defocus the YAG pulse to provide a beam diameter of ~750 μm. The resulting peak intensity of 400 MW cm⁻² is below threshold for any stimulated processes in this system. Both sets of spectra were taken in a rotating sample cell cooled externally by flowing cold nitrogen gas. For the pulsed spectra, these conditions yield a lifetime for the CO-Hb in the laser beam of about 0.3 ns, while for the continuous wave spectra it is estimated to be about 1 ms. Since the latter time is much longer than the time for the rotating cell to turn through the sample volume (~150 μs), the cw spectra represent largely unphotolysed material. In the pulsed case, on the other hand, the first 5% of the pulse was sufficient to photolyse the entire sample. Hence the pulsed spectra represent

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mainly photolysed material within 10 ns after photolysis. Since the rotation time of the cell (100 ms) was much larger than the 10 ms recombination time at 1 atmosphere of CO, each pulse was presented with essentially a fresh sample for study. Each spectrum reported here represents the accumulation of 1,600 or more laser pulses.

The essential results are demonstrated by the spectra shown in Fig. 1. The cw spectrum (Fig. 1a) shows the strong anomalously polarised $1,585\text{ cm}^{-1}$ peak characteristic of low spin haem¹¹. The pulsed spectra of CO-Hb (Fig. 1b) obtained with the entire 10 ns pulse, exhibit the disappearance of this line, and the appearance of another strong feature in the region $1,545\text{--}1,555\text{ cm}^{-1}$. This feature seems to consist of two unresolved lines, similar to the spectrum of deoxy-Hb taken in the same conditions. Spectra on a sample of oxy-Hb showed little evidence of photolysis, as expected. The anomalously depolarised component of the doublet (B) is displayed more accurately by subtraction of the weighted polarised spectrum (VV) from the depolarised (VH), using the fact that the depolarisation ratio for the lower member of the doublet is 0.75, by symmetry. Typical results are shown in Fig. 1c. The observed peak positions are tabulated in Table 1. The line B has been found to be correlated

Table 1 Observed line positions (cm^{-1})

Sample	A (dp)	B (ap)	C (dp)
CO-Hb (cw)		$1,585 \pm 2$	$1,634 \pm 2$
CO-Hb (pulsed)	$1,544 \pm 1$	$1,554 \pm 1$	$1,601 \pm 1$
Deoxy-Hb (pulsed)	$1,547 \pm 1$	$1,556 \pm 1$	$1,606.5 \pm 1$
Oxy-Hb (pulsed)		$1,587 \pm 3$	$1,640 \pm 3$

with porphinato core size. Hence, the spectra shown demonstrate that the major change in core size occurs in less than 10 ns after photolysis. By the use of the Kerr cell it was possible to observe separately the scattering from the first and second

halves of the 10 ns laser pulse. These spectra, although noisier than those shown in Fig. 1, were essentially the same, clearly showing that the main change in the porphinato core size occurs within 5 ns of photolysis. Hence, this reorganisation is completed well before the 100 ns lifetime of the transient observed in ref. 1.

The better quality of the spectra obtained with the full 10 ns pulse, shown in Fig. 1, allows the further conclusion that definite (small) shifts of all three lines are observed between the pulsed spectra of CO-Hb and deoxy-Hb. In the case of CO-Hb, the process is initiated with the haem in the *R* configuration, while the deoxy-Hb it is in *T*. The *R*–*T* transition occurs on a much longer time scale¹. Hence, the small differences observed may be associated with the changes in haem geometry during the *R*–*T* transition. However, we should note the possible existence of a long-lived electronically excited state⁶ in either case (or both) which might itself cause perturbations of the Raman frequencies. Further, the correlation observed between core size and Raman frequency⁹ shows a scatter of $4\text{--}5\text{ cm}^{-1}$. Therefore, the shifts we observe, although apparently indicative of a change of $0.02\text{ \AA}$ in the core size, may instead be the result of the protein reorganisation. Therefore, we cannot state conclusively on the basis of our spectra that a change in haem geometry is associated with the *R*–*T* transition.

As has been noted by others¹², the observation that the core size changes after photolysis does not necessarily imply a movement of the Fe ion out of the plane. Possible distortions of the haem from a planar geometry may also be involved, thus complicating this portion of the structural analysis. The use of different probe frequencies to achieve resonance with various electronic transitions, may eventually be of utility in resolving this point.

To summarise, we have shown that within 5 ns of photolysis, the core size in CO-Hb assumes a value closely approximating that in deoxy-Hb in the steady state. The observed Raman peaks for the transient species are slightly shifted from those of *T* deoxy-Hb. It seems possible that this corresponds to a transient core size about $0.02\text{ \AA}$ larger than that of *T* deoxy-Hb. However, it is not possible to relate these differences more definitely to structural modifications. Moreover, we cannot yet rule out the possibility that additional changes, perhaps non-monotonic ones, in the spectrum may occur over a longer time scale, yet before the *R*–*T* transition. To investigate the possibility of the occurrence of such movement, we plan to use a double-pulse technique to extend this investigation to a wider range of times. Further, a transient Raman investigation of the entire *R*–*T* transition is clearly called for, making use of the variety of resonances available to probe other vibrational excitations.

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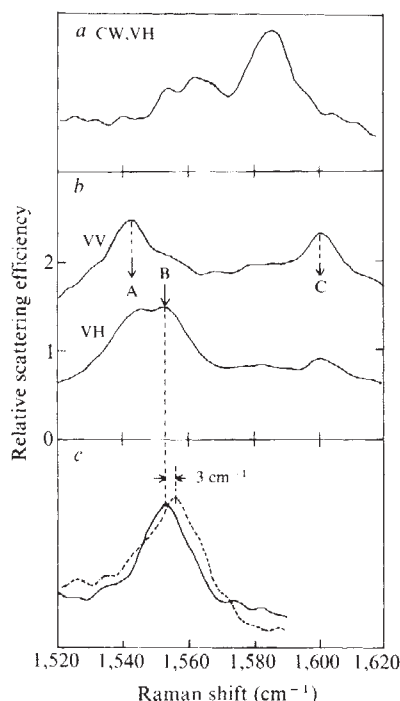


Fig. 1 a, Perpendicular polarised cw spectrum of CO-Hb, obtained with $5,308\text{ \AA}$ excitation, at low power. b, Parallel (VV) and perpendicular (VH) polarised transient spectra of photolysed CO-Hb. c, The solid line represents the anomalously polarised component of the transient, obtained by subtraction of the VV spectrum in (b), multiplied by 0.75, from the VH spectrum. The dashed line represents the result of the same analysis for the pulsed spectra of deoxy-Hb.

matters arising

On melting icebergs

HUPPERT AND TURNER'S laboratory studies¹ of ice melt in warm, salt-stratified water, which demonstrate a system of tilted convection layers of melt mixture interleaved with ambient fluid, are useful in focusing research upon the complex phenomenon of iceberg melt but can not be extrapolated so directly to *in situ* Antarctic iceberg melting as the authors have done, for several reasons: (1) density profile data corresponding to the *T, S* profiles of the Weddell Sea station² shown in Huppert and Turner's article are the depth ranges (and the corresponding density gradients)³: 30–50 m ($1.6 \times 10^{-7} \text{ g cm}^{-3} \text{ cm}^{-1}$); 50–100 m ($1.5 \times 10^{-8} \text{ g cm}^{-3} \text{ cm}^{-1}$); 100–300 m ($2 \times 10^{-9} \text{ g cm}^{-3} \text{ cm}^{-1}$); laboratory study¹ ($1-6 \times 10^{-3} \text{ g cm}^{-3} \text{ cm}^{-1}$); as the mean density gradient has a major influence on the vertical scales of free convection it is difficult to see how laboratory results can describe the oceanic iceberg case where this parameter is from 10,000 to 1,000,000 times smaller. In fact, the density difference over the typical 1 cm vertical scale of a single layer in the laboratory study is six times larger than the total difference in density from 30–150 m of the Central Weddell Sea station. (2) At the 23 °C temperature of Huppert and Turner's salt-stratified laboratory experiment, the thermal expansion coefficient is $0.17 \times 10^{-3} \text{ g cm}^{-3} \text{ }^{\circ}\text{C}^{-1}$; in typical Antarctic waters it is 0.05×10^{-3} , less than one-third as effective in controlling density changes relative to the saline contraction factor which is much the same in both cases (0.81×10^{-3} as opposed to $0.76 \times 10^{-3} \text{ g cm}^{-3} \text{ p.p.t.}^{-1}$, respectively). Furthermore, ambient water temperature in Antarctic water is only about 2 °C above freezing point, an order of magnitude different from their laboratory case. The overall thermal effect on density is then between one and two orders magnitude less in the Antarctic situation.

(3) Antarctic icebergs contain some 7% by volume of compressed air^{4,5}. Effects of air bubble release with melt water at depth are unknown but must be important in modifying vertical convection and horizontal heat diffusion across the boundary layer. A characteristic hissing sound has been reported near icebergs (Seelye Martin, personal communication).

(4) The tilted convection layers observed by Huppert and Turner occur at vertical increments of 1 cm and $2.5 \times 10^{-3} \text{ g cm}^{-3}$ density difference. It required the available heat in 3.5 g ambient fluid to melt 1 g of ice; complete mixing would

result in a mixture of density about $5.6 \times 10^{-3} \text{ g cm}^{-3}$ less than that of the ambient fluid supplying the heat. The characteristic vertical scale of layers ought to have been $>2 \text{ cm}$; it was not, which indicates that some melt heat was supplied by means other than turbulent diffusion, such as, double-diffusion. In the Antarctic iceberg case, for reasons (1–3), turbulent diffusion should dominate over other transfer mechanisms. While field measurements are lacking, data reported by Matthews and Quinlan⁶ for Muir Inlet, Alaska, showed that late winter modification of the bay water by glacier melt followed a 21:1 ratio. The ambient fluid at 3 °C and 31‰ salinity supplied about 96 cal g^{-1} of icemelt and part of this must have been needed to raise the ice temperature to melt point. Thus from the only available field data it seems that ice melt entrains ambient fluid with a ratio governed at a minimum by the heat available through complete mixing with ambient fluid, and not less as in the laboratory results¹.

(5) Step structure observed in Central Weddell Sea profiles cannot be described as a plausible extension of the laboratory results. The thickest layer in the given profile extends from 100 to 200 m with a total density difference of $2 \times 10^{-5} \text{ g cm}^{-3}$; a 1,250:1 mixing ratio is required to alter 1 g of melt water to ambient fluid density during its rise through this vertical distance, and the 2,250 cal of available heat in the entrained fluid far exceeds that needed for melting.

A plausible extrapolation of the laboratory results of Huppert and Turner to the melting iceberg would state that the berg generates one such convective layer. A single cell convection was the basis of my earlier study³ on upwelling by icebergs. Even so, the existence of low horizontal temperature and vertical density gradients, compressed air bubbles in glacier ice and reduced thermal expansion effects, and velocity shear associated with berg motion severely limit the role of double-diffusion in iceberg melting (an exception might be the melting process of the bottom surface of the berg).

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Kerogen maturation and petroleum generation

UJIIÉ¹ concluded that a one-point reversal in the C–H–O maturation pathway of kerogens of the Cretaceous of the Douala Basin indicated that a large amount of hydrocarbons was released from the kerogen. Other interpretations are possible. The basic problem is whether the anomaly reflects hydrocarbon release from similar kerogens, or indicates a change in the dominant kerogen type. The latter interpretation is appealing for three reasons.

(1) The anomalous point is not (*h*) which lies directly on the projections of the maturation pathways defined by the shallower points (*a–f*) and the deeper points (*i–n*). The anomalous point is (*g*) which has higher C and H and lower O contents than adjacent points. The kerogen at (*g*) is near peak oil generation². If the original kerogen types at (*f*) and (*g*) were essentially identical, the H content of the kerogen should be decreasing with depth, not increasing³. The increase in H content with depth from (*f*) to (*g*) indicates an increasing hydrocarbon generating capacity with depth, and suggests a corresponding change in original kerogen type.

(2) By themselves, the C–H–O relationships between points (*f, g, h*) could be interpreted as primarily CO₂ generation from (*f*) to (*g*) and hydrocarbon generation from (*g*) to (*h*). This explanation is unlikely. Pyrolysis experiments indicate that CO₂ generation is greatly diminished by the time significant hydrocarbon generation begins⁴, as it has at (*f*). In addition, thermogravimetric analyses of the (*f*) and (*g*) kerogens indicate that (*g*) had a 50% greater weight loss at 500 °C than did (*f*)⁵. If (*f*) lost CO₂ and reached the composition of (*g*), the weight loss differential would be in the opposite direction.

(3) Kerogen is extracted organic matter. Its C–H–O contents should be identical at the same stage of thermal alteration whether the generated products remained in the kerogen in the subsurface or whether they were released in the subsurface. By itself, kerogen analysis yields no information on hydrocarbon release.

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UJIIÉ REPLIES—The Cretaceous kerogen of the Douala Basin is the best material available to provide a corroborative evidence of my hypothesis on the reversal in the C–H–O maturation pathway of kerogen, because the Cretaceous is composed of the thick argillaceous sedimentary series in which the original organic matter is identical throughout the whole cores taken between 700 and 4,000 m in depth^{1,2}.

Jones concluded that the anomalous point (g) had a different type kerogen. A series of geochemical studies on the organic matter of the Douala Basin indicates the same kind of organic matter provided in the sample (g) as in the other ones^{1–3}. Tissot *et al.*⁴ pointed out that the whole evolution path of these kerogens is the typical example of their 'type III'.

In order to examine the original kerogen type the maceral components of kerogen should be discussed, instead of the minor difference of hydrogen content and weight loss by pyrolysis as was stated by Jones. The maceral component of each kerogen of the Douala Basin almost consist of more than 80% of amorphous organic cement optically close to vitrinite¹, indicating the homogeneous components throughout the whole cores. Therefore, the C–H–O component path from (g) to (h) should be explained by a large amount of hydrocarbon release, not by the difference of original kerogen type in (g).

Kerogen is extracted organic matter. Its C–H–O content should be related to what kinds of the generated products are released from it, if the original kerogen is identical throughout the strata. The same reversal in the C–H–O maturation pathway as shown from (g) to (h) in the Douala Basin is observed in the Miocene argillaceous sediments of the MITI Borehole in Northern Hokkaido. Examination of hydrocarbon-generating capacity by pyrolysis supports that the relative decrease of carbon content is due to the hydrocarbon release in the MITI Borehole samples (data to be published elsewhere). Therefore, kerogen analysis could give us much information on petroleum generation. To determine whether my hypothesis is applicable to the Douala Basin, the most important work to be done is to decide whether the weight loss by pyrolysis at (g) is due to the generation mainly of hydrocarbon or mainly of CO₂ and/or H₂O.

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Stability problem of Kirkwood Gaps

KIANG noticed¹ the importance of the Kirkwood Gaps (particularly the Hecuba Gap) in contrast to Hilda Group in the asteroidal distribution problem, and presented a solution to this problem using a stability criterion inherited in Hill's equation which, he assumed, clarifies the difference between gaps and groups (less populated against populated regions). His discussion, however, discloses the curious fact that he has omitted the first two terms in RHS of his (linearised) variational equation (6), without any convincing ground. This omission causes a critical deviation from the original problem.

Kiang's discussion starts essentially from the canonical equations of motion with one degree of freedom,

$$\frac{dx}{dt} = F_y \quad \text{and} \quad \frac{dy}{dt} = -F_x \quad (1)$$

where the subscripts refer to the partial derivatives with respect to the variables x and y , respectively. Let $x(t)$ and $y(t)$ be periodic. Then he deduces the variational equations,

$$\left. \begin{aligned} \frac{d\xi}{dt} &= F_{yx}\xi + F_{yy}\eta \\ \frac{d\eta}{dt} &= -F_{xx}\xi - F_{xy}\eta \end{aligned} \right\} \quad (2)$$

where ξ and η are the virtual variations to x and y coordinates, respectively.

He insists that from these variational equations he obtained a second order differential equation of Hill's type. Curiously enough, however, the system of equations (2) can be solved by quadrature as will be seen by equations (9), as it has a (variational) energy integral (see also ref. 2)

$$\mu \equiv F_x\xi + F_y\eta = c_1 = \text{constant} \quad (3)$$

This μ is a variation across the orbit. We also have a variation along the orbit by a form,

$$\nu \equiv F_y\xi - F_x\eta \quad (4)$$

which is perpendicular to μ direction. The differential equation for ν can be obtained by a straightforward manipulation as follows:

$$\frac{d\nu}{dt} = (A\mu + B\nu)/D \quad (5)$$

where

$$\left. \begin{aligned} A &= (F_{xx} - F_{yy})(F_x^2 - F_y^2) + 4F_{xy}F_xF_y \\ B &= -2F_{xy}(F_x^2 - F_y^2) + 2(F_{xx} - F_{yy})F_xF_y \end{aligned} \right\} \quad (6)$$

and

$$D = F_x^2 + F_y^2 \neq 0$$

On the other hand, we see

$$\frac{dD}{dt} = B \quad (7)$$

Therefore, we can obtain the solution for ν by quadrature, considering that μ is given

by equation (3),

$$\nu = D[c_2 + c_1 \int_0^t D^{-2} A dt] \quad (8)$$

c_2 being another constant. The solutions for ξ and η are readily given by

$$\left. \begin{aligned} \xi &= c_1 F_x D^{-1} + F_y [c_2 + c_1 \int_0^t D^{-2} A dt] \\ \eta &= c_1 F_y D^{-1} - F_x [c_2 + c_1 \int_0^t D^{-2} A dt] \end{aligned} \right\} \quad (9)$$

Note that the solutions by quadrature do not include a new period as in the case of Hill's equation, although the solutions include the mixed secular term, $t \times$ (periodic term) in general. This situation comes from the change of period according as different energy constants (the energy difference being c_1).

Kiang's argument is totally wrong in the point: he only took into account some of the necessary terms without any convincing ground, so that the equation becomes the Hill's type. In reality, the variational equations (2) can be solved by quadrature, because the system has an energy integral. There is no need to introduce a transcendental method, or a new period. In other words, the stability criterion, with which he wanted to clarify the difference between Hecuba and Hilda cases, is not inherent in the variational equations (2) *ab initio*. Thus the problem is still open.

S. AOKI

Tokyo Astronomical Observatory,
Mitaka, Tokyo,
Japan

1. Kiang, T. *Nature* **273**, 734–736 (1978).
2. Diliberto, S. P. in *Contribution to the Theory of Non-linear Oscillations* Vol. 1 (ed. Lefschetz, S.) 1–38 (Princeton University Press, 1930).

KIANG REPLIES—Aoki's attitude is the conventional one and is responsible for the lack of success in solving the Kirkwood Gaps problem so far. He apparently believes that because he could write down the solution of the basic equations by the classical method, there is no need to introduce other methods. But the classical method is, in principle, incapable of solving the problem at hand, because it always leads to results (see, for example, Aoki's equations 9) that apply to all resonances equally—it can never explain the Hecuba–Hilda difference. Hence I regard it highly desirable to introduce some new method, equivalent to more or less radical departures from the classical method. Some modification to explain the Hecuba–Hilda difference within the frame of the simplest gravitational model is necessary but as to the form of modification I adopted in my paper, I made it clear that there may well be grounds for improvement. I do not expect any improvement that I could give here to substantially alter the result obtained.

T. KIANG

Dunsink Observatory,
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reviews

Wright's late works

Alan Robertson

Evolution and the Genetics of Populations. Vol. 4: Variability within and among Natural Populations. By Sewall Wright. (University of Chicago: Chicago and London, 1978.) \$46.90; £26.25.

VERDI wrote *Otello* when he was 73 and completed *Falstaff* at the age of 80, though twenty years earlier he had produced some minor works like *Trovatore* and *Rigoletto*. Sewall Wright, though he had contributed extensively to the scientific literature since 1915, published his first book, the first volume in this series of four, in 1968 at the age of 78 and the final two have now appeared in quick succession. The first laid the biometrical and genetic foundations and the second, almost entirely theoretical, extended these to the population level in terms of gene frequencies and concluded with a section on quantitative genetics. The third volume was to discuss the evidence but while the first two were being written so much new evidence became available that it has now become two—the first dealing primarily with evidence from laboratory experimentation and the present one with that from natural populations. The new information came mostly from two new techniques. The first of these, starch gel electrophoresis, has not only produced a great deal of information for the study of population structures (as can be seen from the present contents of journals like *Evolution*) but also answered a previously unanswerable question: What proportion of loci are segregating in wild populations? (the answer is, however, restricted to those loci specifying peptide chains). The second kind of information, from amino acid sequences, can provide a direct genetic measure of the rate of evolutionary substitution at such loci.

Wright's general approach in these final volumes is to analyse specific papers in some detail (sometimes with a re-analysis of his own). For instance, he deals with some work from the early days of genetics like that of Castle on hooded rats (Wright was a junior author of the paper in 1916) and Sumner's work on *Peromyscus*, but also discusses repetitive sequences in DNA and treats extensively the evidence from biochemical polymorphisms in American *Drosophila* populations from Ayala and his colleagues. The volumes

form a valuable reference work and provide a source of critical discussion.

Wright is the last of the three exponents of mathematical studies of evolution made up of himself, Fisher and Haldane, and the final chapters give his summing up of the present situation. The three, working almost independently over about forty years, were very different in temperament and in training. Fisher and Haldane had degrees in mathematics from Cambridge and Oxford, respectively; Wright had a degree in Zoology from a small mid-Western college and continued his experimental work with guinea pigs until he retired from Chicago. Nevertheless, he has always shown extraordinary mathematical insight in solving the problems that he encountered. In fact, a paper of his in 1915 may be considered as foreshadowing the invention of analysis of variance.

He has, I think, always felt that his ideas suffered from a simplistic representation by Fisher and his colleagues. I remember him saying once that "The position got complicated by the appearance of another person in the argument, with the same name as myself but holding completely different views". He felt himself saddled with a naïve outlook about the importance of sudden reductions in population size leading to "genetic drift". He was a little surprised to have some British authors scorn "classical random drift" while espousing the "founder principle" which he thought of as a special case of the former. "While it is useful to make such distinctions, it is evident that most of those who have done so have failed to grasp the general principle of effective size in relation to random drift". When Mayr made his criticism of "bean bag genetics" at Cold Spring Harbor in 1959, Haldane felt it necessary to write in reply; Wright did not, he had always taken a more sophisticated view. He returns to the differences in approach on page 464 of the final volume. "It is sometimes supposed, following Mayr (1959), that the early mathematical studies of evolution under Mendelian heredity by Haldane, Fisher and myself were all essentially equivalent, being based on the same simplified biological premises: populations almost homallelic with respect to an array of

'wildtype' genes except for rare favourable mutations, fixed one at a time, by mass selection, the model referred to by Mayr as 'bean bag' evolution. Actually the processes on which we put major emphasis [Haldane on the basis of major mutations; Fisher on the basis of multiple, largely minor mutations; Wright on the basis of local peak-shifts among interaction systems] were about as different as possible, under the common assumption of Mendelian heredity. Each, however, might be valid for particular characters under particular conditions in species with particular population structures".

His view of the importance of periods of small local population size was not so much that this could of itself lead to fixation in a species but rather that, in an interacting system, such chance changes in gene frequency could allow the population to move, as a consequence of the subsequent selection, from one adaptive peak to another. As with all models involving interactions between many loci, it is not amenable to direct algebraic treatment and Wright's approach has been mainly verbal, dealing numerically with specific examples.

In the past decade the old arguments took on new life in the 'neutralist-selectionist' controversy about the mechanisms of the maintenance of genetic variation within populations (the evidence coming mostly from electrophoresis), and about the rate of gene substitution in protein evolution. Ironically, the neutralist view corresponded somewhat with the more simplistic versions of his views which he had rejected so indignantly in the 1930s. From his discussion on both these points, it is clear that he is in essence a 'selectionist', though he is writing before the recent findings that modifications of electrophoretic technique could increase greatly, at least for some loci in *Drosophila*, the number of alleles known to be segregating in populations.

The fascination of these final volumes comes from the breadth of historical perspective: for the greater part of the development of modern genetics, Wright was really there. □

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Metal sulphides

Mineral Chemistry of Metal Sulfides. By D. J. Vaughan and J. R. Craig. Pp. 493. (Cambridge University Press: Cambridge and London, 1978.) £19.50.

METAL SULPHIDES are not only of concern to geologists in economic, petrological and sedimentological fields but also to metallurgists in many specialisms from the extractive industries to electronics. This book aims to lead all such workers through a variety of experimental and theoretical approaches into a clearer understanding of the range of natural and synthetic sulphides. The authors have made individual contributions to sulphide research and now they have assimilated a vast literature to produce a concise and balanced book.

The text and appendices contain an immense amount of well indexed data with generous references to original work; this will therefore be a satisfying handbook to investigators, but not a manual of experimental techniques. Vaughan and Craig, however, seem to have had the additional objective of teaching the willing reader who might be ignorant of one or other of the particular subjects covered, from crystal chemistry to determinative

methods. In the introduction and elsewhere the underlying theory is introduced straightforwardly and without condescension.

Only four years after P. H. Ribbe's *Short Course Notes* on sulphide mineralogy (Mineralogical Society of America, 1974) it may be asked "why another publication on the same ground?". Although the aims and constraints are here somewhat different, one answer might be that Ribbe showed it could be done. Until that date the variety and complexity of the sulphides defied condensation; their classification was never as straightforward as that of the silicates, and only with the recent deployment of crystal chemistry to the explanation of sulphide structures has a more satisfying rationalisation been glimpsed. Sulphosalts will perhaps be the last to emerge from the jungle but anyone concerned with these not necessarily rarer variants will still have to rely on the reviews of Ribbe's contributors, as Vaughan and Craig dismiss them entirely in order to limit the size of their text.

Naturally this volume is altogether more polished. On the whole, it parallels the 1974 book on sulphide crystal chemistry and classification, with some later work to 1977 included,

and also on chemical bonding, principal phase equilibria of sulphides and associated experimental methods, and current trends and prospects in the investigation of natural sulphides in geology.

Part of the distinctive trend of the book is found in the balancing chapters on electrical and magnetic properties and their applications, spectroscopic studies, classical (that is, including microscopic) determinative methods and sulphide thermochemistry. The chapter on sulphide equilibria in aqueous solutions leads to consideration of conditions in natural hydrothermal fluids and to the thermodynamics of sulphide solubility and deposition.

The final chapter discusses natural sulphides and recognises their differences from the pure and synthetic in their major, minor and trace element contents, solid solution phenomena and S-isotopic variation, the study and significance of all of which are introduced. Appendices contain mineralogical data on 319 sulphides, univariant sulphidation data and invariant points in sulphide mineral systems.

L. G. Love

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Heterogeneous catalysis

Dynamic Heterogeneous Catalysis. By K. Tamaru. Pp. 140. (Academic: London and New York, 1978) £8.50.

It is difficult to know for whom this volume is intended. Its objective is to emphasise that an understanding of heterogeneous catalysis cannot be achieved by studies of the equilibrium states of solid/gas systems, but must be based on a study not only of the kinetics of catalysed reactions but also of the changes in adsorption on, and chemical properties of, the catalytic sites under working conditions. This thesis is widely accepted and has indeed been the objective of much research, particularly by the author and his coworkers. The complementary view, that one must first understand the nature of clean surfaces and their interaction with simple gases, is, however, implied by a great deal of recent work using modern techniques of surface physics, so that an attempt to strike a balance between the two would be timely.

One therefore expects this to be a research monograph. Instead, one finds a very uneven text including a great deal of elementary physical chemistry (for example, kinetic derivation of the

Langmuir isotherm, Langmuir-Hinshelwood kinetics, and a discussion of homogeneous gas reactions) with which the average research worker in the field will be very familiar. Yet the presentation is not sufficiently balanced to serve as a supplementary undergraduate text. The descriptions of various surface physics techniques—FEM, FIM, LEED, ELS, AES and PES—are very sketchy and hardly seem justified as only scant reference is made to them later in the book: again the research worker in this field will already be very familiar with these techniques.

The book consists of four chapters on: general reaction kinetics; adsorption; the kinetics of heterogeneous reactions; and the applications of spectroscopic techniques to the dynamic treatment of adsorbed species in reaction conditions. Only in the last chapter, in which the examples are drawn largely from the author's own work, does the main theme of the book begin to dominate.

Libraries carrying a comprehensive collection on catalysis will no doubt wish to have this book, but it is unlikely to attract a wide readership.

D. H. Everett

D. H. Everett is Leverhulme Professor of Physical Chemistry at the University of Bristol, UK.

Understanding living systems

Ordre et Dynamique du Vivant: Chemins de la Biologie Moléculaire. By A. Danchin. Pp. 381. (Editions du Seuil: Paris, 1978.)

THE object of this book, as specified by the author himself, is to emphasise the contribution of our knowledge of molecular phenomena to the understanding of a living system as a whole, including the human brain processes. The book opposes those theories which purport that our understanding of the way a whole organism is working is a specific property of the system and cannot result from the analytical study of each of its components, and concludes that the fundamental properties of a living system are the direct consequences of the properties of its constitutive molecules.

The author chooses several "reduction levels". He first examines the general characteristics of a cell and the modalities of its development. He then considers a second reduction level, the chromosomal one, and includes the rudiments of genetics and indispensable notions on cellular division. In the third and most extensive part, he comes to the fundamental reduction level, the molecular one. After recounting the nature of the interactions between atoms and between molecules, he gives a clear account of the processes of molecular biology—that is, an account of the mechanism of protein synthesis and its regulation—and has succeeded very well in describing the basis of these processes clearly and concisely.

The last part is the most original. It begins with an examination of the nature of viruses and an analysis of the possibility of their synthesis, continues with a study of genetical manipulation experiments already accomplished or to be considered in the future, and ends with an examination of the feasibility of hypotheses concerning the origin of life and the evolution of species. He explains the relationship between phylogenesis and variations in the amino acid sequences of proteins and why he conceives the evolution of species as the development of controls: some fluctuations in the hereditary programme favour the adjustment of the system to external conditions and are therefore integrated in the descendants. There must be a limit, however, to what can be recorded in a genetical programme, and non-hereditary modifications will occur. These individual processes, which the author calls epigenesis, allow adjustment to the external medium. The epigenesis of the nervous network is given as a good example.

This book is aimed to non-specialists. My main regret is that the author's care in explaining clearly the physico-chemical mechanisms of the biological processes to non-initiates did not allow him to develop his own ideas more extensively. Nevertheless, this interesting book succeeds well in its stated purpose of showing that the working

of living systems can be explained by the behaviour of their constitutive molecules and their appearance by physicochemical phenomena.

J. Tonnelat

J. Tonnelat is Professor of Physicochemical Biology at the Université de Paris-Sud, Orsay, France.

Regulators of plant growth

Natural Plant Growth Inhibitors and Phytohormones. By V. I. Kefeli. Pp. 278. (Junk: The Hague and Boston, 1978.) Dfl.90; \$44.

THE known and accepted hormonal regulators of plant growth and development comprise the auxins, the gibberellins, the cytokinins, abscisic acid and (perhaps) ethylene. Each of these substances has a clearly defined range of physiological activities, and it is the interplay of these activities which results in the coordination and regulation of the various processes and development. Some plant physiologists, however, believe that this is not enough, asserting that the precise regulation of a process may only be achieved through a balance between stimulatory and inhibitory actions. Given this premise, it is inescapable logic that the auxins, gibberellins and cytokinins, whose actions on cell growth and differentiation are predominantly stimulatory, should be balanced by endogenous, growth-inhibitory substances. As almost any substance, at sufficiently high concentration, will inhibit plant growth, it is obviously very easy to find such inhibitors; it is, on the other hand, and equally obviously, extremely difficult to prove that such growth-inhibitory substances actually act, endogenously, to inhibit growth. Dr Kefeli is a protagonist of the inhibitor camp, and he uses this book in a forceful and single-minded attempt to convince the reader that his view is correct.

The book is based on the author's work carried out at the Timiryazev Institute of Plant Physiology of the USSR Academy of Sciences between 1961 and 1967, and its most valuable contribution lies in providing, for Western scientists, in English, a readable and well documented summary of work which is not easily available from published literature. It is also useful in bringing together diverse information on the synthesis and hypothetical functions of growth-inhibitory substances.

There are, however, many shortcom-

ings. The background literature is only referred to when the conclusions favour the case being made. Also, doctrines which are currently under question in the West (for example, the Cholodny-Went theory of tropisms, the gene expression theory of phytochrome action, and the presumed control of transcription by auxins and gibberellins) are presented as established fact. Critical analysis of data is practically non-existent. This book seeks to make a case, not to present a reasoned and unbiased account. Thus, only the reader himself can judge whether it is successful. Personally I remain unconvinced.

H. Smith

H. Smith is Professor of Plant Physiology at the University of Nottingham, UK.

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Laboratory power supply. Buchler. A laboratory power supply featuring an L.E.D. digital display, has been introduced by Buchler Instruments. The display shows watts, milliamperes or volts, and can be easily read. Maximum outputs are 1,000 V, 200 mA and 200 W. The unit can operate in either a constant-voltage, constant-current, or constant-power mode, instantly selected by pushbutton. Overvoltage and over-current protection are built in. The unit is particularly designed for the needs of workers in research and clinical electrophoresis. Voltage and current regulation are 0.1% and 0.2%, respectively.

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Solid state digital store. Wayne Kerr. Wayne Kerr have introduced the ADS1 solid-state digital store to complement their RA200 AF response analyser. The RA200/ADS1 provides a continuous response curve, continuously updated with no chance of missing a sharp spike at some point, and no internal filters or integrators to distort the response pattern. Whether the signal source is the built-in sweep oscillator, or a gliding-tone test tape or disk, the dBs can be read from the vertical scale and frequency from the logarithmic horizontal scale. The solid-state digital store will record up to four complete curves and retain the information (with the RA200 switched off) for 2 weeks.

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The Reichert-Jung Abbe Refractometer

Spectrophotometer. Erba. The new Carlo Erba UV-vis spectrophotometer, the Spectracomp 601, allows all information to be keyed in through a simple keyboard. The spectrophotometer incorporates a programmable computer, offers custom-made programs of analysis, and gives a clear print-out of all instruments' parameters. It allows statistical analysis of spectral data, incorporates a real time clock and a print-out of time, date and temperature, and graphically displays values of absorbance and transmittance versus wavelength on an incremental plotter.

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Circle No. 60 on Reader Enquiry Card.

obituary

Michel Magat

MICHEL MAGAT, professor at the Paris-Sud University of Orsay, died on 6 June 1978. He had been for more than forty years one of the notable physical chemists in Europe.

Born in Kharkov in October 1908, he did his first research in Germany with Ernst Bergman (who was to become one of the organisers of chemical research in Israel) before joining the group of Edmond Bauer at the Sorbonne. There some of his publications made him widely known before he had reached the age of thirty. During the war he was with the Free French Forces until 1945. He then spent a long time at Princeton, adding to his already wide range of interests that of polymeric materials and their behaviour under intense irradiation.

After the war he was among those whom Frédéric Joliot—at the time head of the Centre national de la Recherche scientifique—entrusted with the development of French physico-chemical research. Much of what has been achieved since has been due to him and to his many brilliant pupils. Director of research at the C.N.R.S., he later became assistant professor at the Sorbonne, then full professor. When a Faculty of Science was established at Orsay, he was among the first to go there, and contributed greatly to make it one of the liveliest research centres in France.

He always showed a great interest in his teaching, and in coordinating his lectures with those given on neighbouring subjects. In this too he was a precursor of what has since become commonplace. He was one of those who were not afraid to state that doing good research did not always qualify one to be a teacher.

Radiation effects, polymers, molecular collisions, relaxation phenomena, reaction transients, nearly all aspects of molecular spectroscopy, the structure and behaviour of water, liquids and glasses were among the subjects this encyclopaedic man took up with constant success. His principal concern was fundamental research but he kept a keen sense of realities, and a number of his studies have had useful applications. Graduates came to his laboratory from many countries to benefit from his direction. Throughout the world, his many former students kept with their former 'maitre' the ties of friendship, and often looked for the advice and judgment of his experience and

fertile imagination—the more so as he was a true internationalist, always concerned with improving scientific and cultural exchanges, promoting collaborations and cooperations across frontiers, and also across intellectual and political barriers.

Magat had been president of the Société de Chimie physique, which had recently made him its honorary life president. He was also a vice-president of the Faraday Division of the Chemical Society, and a scientific advisor of the Hahn-Meitner Institute in Berlin. His many travels, his remarkable fluency in many languages, his interest in man and his sense of humour also made him a living repository of the world's folklore and jokes.

Many colleagues and friends mourn his death. Michel Magat achieved much in his research and teaching. He was also fully committed to every cause and principle in which he believed. Age, experience, fame and responsibilities had not dulled in him the sense of justice and duty. He had the friendship and respect of all those who knew him.

C. Troyanowsky

W. D. Chesterman

PROFESSOR W. D. CHESTERMAN, distinguished for work on high speed photography and side-scan sonar, died on 6 July 1978 at the age of 65, after a short illness.

After graduating at Bristol University in 1934 he worked in the research department of the British Thomson-Houston Company. In 1939 his industrial experience proved of value to the Admiralty at the Signal School in Bristol and in 1943 at H.M.S. Vernon. In 1945 he was in charge of the Admiralty Photographic and Instrument Research Laboratory and then moved to the Admiralty Research Laboratory, Teddington.

He was much concerned with the development of techniques involving photography and, in a joint study with industry, made a major contribution to the design of a high speed camera. An underwater study, combining theory and experiment (with short period flash and high speed photography), gave an elegant demonstration of the changing forms of transient, but troublesome, cavitation bubbles.

He became a Fellow of the Institute

of Physics in 1943 and of the Physical Society in 1945. Among his early publications was an authoritative book, on the photographic study of rapid events (1951), which proved of wide interest. His leadership was also recognised by his chairmanship of the International Committee on High Speed Photography (1956–58), and by his Fellowship of the Royal Photographic Society in 1958.

Meanwhile, in 1949 he joined the Admiralty Underwater Weapons Establishment, where his restless enthusiasm and drive stimulated the application of side-scan sonar. He made the first extensive true-plan presentation of sea floor patterns, from an overlapping mosaic of records. The scientific value of the method for showing relief and texture in plan view was illustrated in a joint paper in 1958. In 1959 he was awarded a D.Sc. by Bristol University for his pioneering work in several fields.

In 1960 he made a courageous change by taking the Chair of Physics at Hong Kong. Under his stimulating guidance the department grew in size, became interested in the surrounding seas and attracted visits from other workers. His own research included sonar surveys of nearby sea floors as well as banks of the South China Sea. All was new, but of particular interest was his study of patterned coral floors. He also demonstrated the method in Canadian waters.

In 1966 he became head of the Geophysics Group of Bath University and the Chair of Geophysics was created for him in 1969. He built up the group, attracted funds from outside and encouraged interest in acoustics and the sea. He found a ready demand for side-scan sonar surveys of potential under-sea cable routes and sought to improve search and presentation techniques. Although these aims were commercial the data obtained were scientifically valuable. In 1975 he became head of the Physics School and then became Professor Emeritus just before retirement in 1978. He had been an effective member of University Senate and Council.

His humanity, charm and vivid personality won him many friends and made him easy to work with. These qualities, and his use of hyperbole helped to coax opponents into willing helpers and were valuable aids when handling pomposity. He calmed the nervous students at vivas and had the vital gifts of making young researchers

feel significant and of welding them into an effective team. He had a healthy disrespect for unjustified bureaucracy.

He paid many visits to the mountains and was particularly keen on high level walks. His considerable knowledge and love of music was a pleasure to his friends, an asset to the University Music Society and led to a deep involvement in the management of the Bath Festival. He was largely responsible for the creation of the elegant Crafts Study Centre in Bath.

He leaves his wife Jean and two children Merlyn and Patrick.

A. H. Stride

Vivi Laurent-Täckholm

BIOLOGICAL science in general, and Egyptian botany in particular, has suffered a great loss with the passing of Vivi Laurent-Täckholm, who died in Stockholm on 3 May 1978, at the age of 80.

This grand old lady had been closely associated with the University of Cairo in Giza for over 50 years, some 32 years as a professor of botany in the Herbarium. Vivi Täckholm was recognized and respected not only as the 'dean of Egyptian botany' but appreciated by all who knew her as one of those rare scientist-scholars who is completely selfless. She helped countless students and investigators both in Egypt and abroad by giving freely of her vast experience and deep erudition on all matters Egyptian, motivated only by a sincere hope that a more carefully planned research project could be launched, or if a project was near completion, that a more mature complete interpretation could be achieved.

A brief overview of her life reveals Vivi Täckholm as a very unconventional woman. She was born in Djurs-holm on 7 January 1898. After obtaining her fil. kand. degree in botany (embryology) in 1921 at the Stock-holms Högskolas, she travelled to the U.S.A. where she worked from east coast to west coast at various jobs, including being a servant. She also began at that time her career as a writer, with a two volume work illustrated with her own photographs entitled *Vivi's resa . . .* (Vivi's Journey). This was first published in Stockholm (1923-24) and soon after in German translation (Gotha, 1925-26).

After her return to Sweden, she held a position in the editorial department of the *Nordisk Familjebok* (Nordic Family Book) from 1923-26 (and again in 1931-33). From 1923-25 she also worked as a journalist for various

Stockholm newspapers. She wrote the children's classic *Sagan om Snipp, Snapp, Sorum* (The Saga of Snipp, Snapp, Sorum) during that period (published 1926, last reprinted in 1976.)

In 1926 she married Gunnar Vilhelm Täckholm, a promising botanical investigator who had earned his doctorate at Stockholm in 1922 and had already gained a reputation for his cytological work. Gunnar Täckholm was called as Professor of Botany to the Egyptian University in Cairo (founded in 1908) in September 1925 to found a department of botany in the newly established Faculty of Science. For the next three years, Vivi aided her husband with all those duties associated with running the department (housed in the former harem building of the Za'afaran Palace) and taught the practical botany instruction for students of medicine, pharmacy and dentistry.

During those early years the need for an Egyptian flora was greatly felt, and the plans were laid for the monumental, but still unfinished *Flora of Egypt*. Also a number of major field trips were made and extensive botanical material collected. The Täckholms stayed in Egypt until 1929.

Vivi then spent a year and a half at the Herbarium Boissier in Geneva studying and comparing their specimens with the type specimens of *Flora Orientalis*. She would later acknowledge the importance of this period not only for her understanding of floristics and taxonomy but for the stimulation and encouragement gained from Robert Chodat, the eminent botanist-taxonomist. Vivi Täckholm continued work on identification of Egyptian plants in Berlin, Kew, London, Weimar and Stockholm under scholarships from the Royal Swedish Academy of Sciences. She and her husband continued their collaboration until his death from tuberculosis in 1933.

Vivi Täckholm was persuaded to continue the work her husband and she had started. The Botany Department at Cairo, especially the Herbarium, grew and profited by her association. Under difficult conditions she gradually accomplished wonders. Indeed she called herself 'the world's biggest beggar.' Largely through her labour and efforts, the herbarium grew to be one of the largest and finest in Africa; the same is true of its botanical library.

In 1941, 16 years after its inception, volume I of the *Flora of Egypt* authored by Vivi and Gunnar Täckholm, in collaboration with Mohammed Drar was published as a Cairo University (then Fouad I University) bulletin; volumes II, III and IV appeared in 1950, 1954 and 1966 respectively. These later volumes were co-authored by herself and her Egyp-

tian collaborator and friend Drar. When Drar died in December 1964 at the age of 70, Vivi Täckholm expressed her great concern for the future of the Flora project which she continued to work on until her death.

The *Flora*, not unexpectedly, is an unusual work. It is a labour of love, clearly carried out unhurriedly in the tradition of those great botanical scholars she would emulate. It has been hailed as a model of scholarship and completeness. One special feature is the detailed treatment of the extensive ancient Egyptian tomb flora; another is the attention paid throughout the volumes to agriculturally important plants and economic botany. Also the enormous bibliographies, which provide access to the ancient and modern Arabic literature, are gems. Since the completion of the *Flora* would take many additional years, a version especially aimed at students, was published in 1956 (2nd edition 1973).

In 1952 Stockholms Högskolas conferred upon her an honorary doctorate. In April 1978 she was honored by the Royal Swedish Academy of Sciences with the gold Linné medal for her pioneering research. The Egyptian government bestowed her with high order decorations on her 60th and 70th birthdays. A special Festschrift entitled *50 Years Cairo University Herbarium—80 Years Birthday of Vivi Täckholm* was published in 1977.

In addition to several shorter, research publications, Vivi Täckholm authored numerous popular and semi-popular books. In these works her supreme knowledge of Egypt as well as her deep love for plants, Egypt, and the Egyptian people is apparent. Indeed she is attributed with saying she 'could live without food, but not without flowers.'

Cairo University has recently decided to name the Herbarium in her honor. But the greatest legacy of Vivi Täckholm is the tradition of love for work and scholarship that she set for her many students and associates. She was also gifted with an exceptional and gentle personality. All who knew her, well or slightly, agree that they are better off, even enriched, by having associated with her. Her smile, bright eyes, fluffy snow white hair, generosity and readiness to help will be remembered warmly by many. Those who had the pleasure of visiting her at her apartment in Orman will also recall her gracious hospitality.

Her closest survivors, a brother, Professor Emeritus Torbern Laurent, her sisters Maj Laurent, textile designer and Gavonne Laurent, several nieces and nephews, and their families, are extended our deepest sympathies.

Abraham D. Krikorian